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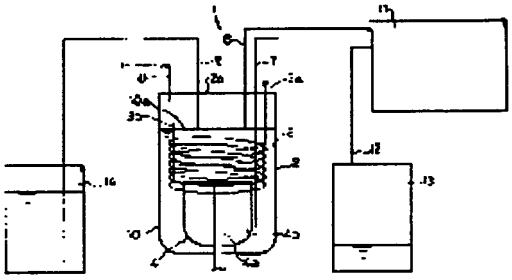
(54) CULTURE METHOD FOR ANIMAL CELL AND DEVICE THEREFOR

(57)Abstract:
PURPOSE: To accomplish mass culture of animal cells in high density without damaging or destroying the cells by agitation under specified conditions using an agitator in a culture tank.
CONSTITUTION: The revolving shaft 4a of an agitator 4 to suspend animal cells into a medium 10 is equipped with plural agitating blades 4b..., and the radius of gyration of each of the agitating blades is set at 1/4 to 3/8 times the inner diameter of a culture tank 2 and the number of revolutions at 5 to 30r.p.m. The cells are fed with oxygen through an aerating tube 3, the cells are separated from the medium using a centrifugal separator 11, and a fresh medium is fed from a medium reservoir 14 into the culture tank.



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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The culture approach of the animal cell characterized by agitating the above-mentioned cell culture liquid with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration it is the culture approach of the animal cell cultivated in the cell culture liquid filled by the cultivation tank, and is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank].
[Claim 2] The culture approach of the animal cell according to claim 1 characterized by making rotational speed into the following by 800cm/in the periphery part of an impeller.
[Claim 3] The culture approach of the animal cell according to claim 1 or 2 characterized by returning an animal cell to a cultivation tank, and supplying new cell culture liquid to a cultivation tank with a culture medium supply means while an oxygen supply means to have a porous tube at least performs tube aeration, and supplying oxygen into cell culture liquid at an animal cell, carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank.
[Claim 4] The culture apparatus of the animal cell characterized by having a churning means to have the impeller formed in magnitude from which the area whose radius of gyration it is the culture apparatus of the animal cell cultivated in the cell culture liquid filled by the cultivation tank, and is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank], and agitating the above-mentioned cell culture liquid with this churning means.
[Claim 5] The culture apparatus of an animal cell according to claim 4 with which rotational speed is characterized by being the following by 800cm/in the periphery part of an impeller.
[Claim 6] The culture apparatus of the animal cell according to claim 4 or 5 characterized by having a separation means to return an animal cell to a cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank while it has a porous tube at least, centrifugal separation of an oxygen supply means to perform tube aeration and to supply oxygen into cell culture liquid at an animal cell, and cell culture liquid and an animal cell is carried out and cell culture liquid is sampled out of a cultivation tank.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] By the continuous culture method, this invention relates to the culture approach of the animal cell which makes this animal cell produce the specified substance, such as a monoclonal antibody, and a culture apparatus while cultivating animal cells, such as an antibody forming cell.
[0002]
[Description of the Prior Art] Establishment of the mass culture approach of the animal cell in a industrial scale is becoming very important as a manufacture means for manufacturing biologicals, such as recent years, for example, interferon, erythropoietin, a colony stimulating factor, and a monoclonal antibody.
[0003] Although culture of an animal cell (a "cell" is only called hereafter) is performed the laboratory-scale which used culture containers, such as a culture flask and a spinner flask, in having scaled up such a small-scale culture approach simply in the industrial scale, it produces un-arranging in respiratory gas exchange, churning effectiveness, etc. of a cell, and cannot carry out mass culture of the cell. Then, the mass culture approach of a cell modeled after the mass culture approach using a microorganism is considered variously conventionally.
[0004] However, cells differ in respect of the following as compared with a microorganism. That is, since ** mechanical strength is weak, a cell tends to receive damage by churning, foaming, etc. of culture medium, as compared with a microorganism, in order to perform ** growth, it needs various nutrients and growth factors, and ** proliferation rate is very slow (doubling-time microorganism: during 0.2 - several hour, and cell: one - several days). For this reason, when mass culture of the cell is carried out using the jar fermenter (churning type culture apparatus) used for the mass culture of a microorganism, cell density cannot be made high in order that growth may stop, when a cell cannot fully grow in response to a mechanical damage etc. and the nutrient in a culture container is consumed. Therefore, when mass culture of the cell is carried out using the mass culture approach of a microorganism, it has the problem that the productive efficiency of the specified substance is very bad.
[0005] Then, in order to solve these problems, the continuous culture approach called high density perfusion cultivation is devised. By supplying the new culture medium containing a nutrient to a cultivation tank, high density perfusion cultivation maintains a cultivation tank at the optimal conditions for growth of a cell, and production of the specified substance, and is extensive and a method of cultivating to high density and producing the specified substance about a cell while it samples the supernatant liquid (supernatant) of cell culture liquid (a culture medium is called hereafter) including the specified substance and wastes out of a cultivation tank.
[0006] As the above-mentioned conventional high density perfusion cultivation, a cell is enclosed with the hollow filament cartridge which has the pore of microscopic smallness, for example. How (JP,62-171669,A) to proliferate a cell by exchanging nutriments etc. through the above-mentioned hollow filament, and to produce the specified substance, Fixed cultivation which fixes a cell to the front face of the support which consists of a ceramic etc., is made to increase a cell by exchanging nutriments etc. in the above-mentioned carrier surface, and produces the specified substance, such as an approach (OPUCHISERU (CHARU sliver, U.S.)), For example, although suspension culture, such as an approach (JP,62-134086,A) of making high density increasing a cell and producing the specified substance by combining the decollator which separates a jar fermenter, and an aforementioned cell and an aforementioned culture medium, is proposed Since the sampling of a cell, quality control of the specified substance, or enlargement of equipment is easy, promising ** of the suspension culture is carried out.
[0007]
[Problem(s) to be Solved by the Invention] However, in suspension culture, in order to maintain a cultivation tank at the optimal conditions for growth of a cell, and production of the specified substance, removal of the physical irritation (shearing force, foaming, etc. by churning of a culture medium) to a cell has been an important technical problem.
[0008] Since churning of a culture medium is performed by rotating the impeller formed in magnitude from which a radius of gyration becomes 1/4 or less [of a cultivation tank bore] at the rotational frequency more than 30r.p.m., a turbulent flow occurs in a culture medium, and it gives physical irritation, such as shearing force, to a cell, and has the problem of having bad influences, such as damage and destruction, on a cell.
[0009] Thus, in the above-mentioned conventional suspension culture, removal of the physical irritation (shearing force, foaming, etc. by churning of a culture medium) to a cell is inadequate. Therefore, it has come to perform mass culture of a cell.
[0010]
[Means for Solving the Problem] Invention-in-this-application persons came to complete this invention, as a result of repeating examination wholeheartedly, in order to solve the above-mentioned technical problem.
[0011] Namely, the culture approach of the animal cell invention according to claim 1 In order to solve the above-mentioned technical problem, it is the culture approach of the animal cell cultivated in the cell culture liquid filled by the cultivation tank. It is characterized by agitating the above-mentioned cell culture liquid with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank].
[0012] The culture approach of the animal cell invention according to claim 2 is characterized by making rotational speed into the following by 800cm/in the periphery part of an impeller in the culture approach of an animal cell according to claim 1, in order to solve the above-mentioned technical problem.

[0013] The culture approach of the animal cell invention according to claim 3 In order to solve the above-mentioned technical problem, while an oxygen supply means to have a porous tube at least performs tube aeration and supplying oxygen into cell culture liquid at an animal cell in the culture approach of an animal cell according to claim 1 or 2 While carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank, it is characterized by returning an animal cell to a cultivation tank, and supplying new cell culture liquid to a cultivation tank with a culture medium supply means.
[0014] The culture apparatus of the animal cell of invention according to claim 4 In order to solve the above-mentioned technical problem, it is the culture apparatus of the animal cell cultivated in the cell culture liquid filled by the cultivation tank. It has a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank]. It is characterized by agitating the above-mentioned cell culture liquid with this churning means.
[0015] In order that the culture apparatus of the animal cell of invention according to claim 5 may solve the above-mentioned technical problem, in the culture apparatus of an animal cell according to claim 4, rotational speed is characterized by 800cm being the following by /in the periphery part of an impeller.
[0016] The culture apparatus of the animal cell of invention according to claim 6 In order to solve the above-mentioned technical problem, it sets to the culture apparatus of an animal cell according to claim 4 or 5. An oxygen supply means to have a porous tube at least, to perform tube aeration into cell culture liquid, and to supply oxygen to an animal cell, While carrying out centrifugal separation of cell culture liquid and the animal cell and sampling cell culture liquid out of a cultivation tank, it is characterized by having a separation means to return an animal cell to a cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank.
[0017] As the above-mentioned culture approach, a suspended-cell-culture method etc. is mentioned, for example, as this suspended-cell-culture method, a batch culture method, a SEDDO batch culture method (a semibatch culture method, fed batch culture), a continuous culture method, etc. are mentioned, for example, and high density perfusion cultivation etc. is mentioned as the above-mentioned continuous culture method, for example.
[0018] Moreover, when performing a continuous culture method as the above-mentioned culture approach, there is the approach of dissociating using ** filter, ** centrifugal separation separating which is made to sediment with gravity using ** sedimentation tubing or a settling tank, and is separated among the separation approaches of a cell and a culture medium. Moreover, when using a Homo sapiens antibody forming cell as an animal cell, the serum-free culture liquid with which 0.1 or more g/l and lactic-acid concentration were adjusted [protein concentration / the dissolved oxygen concentration in 5 or less mg/l and cell culture liquid] for 0.1 ppm or more, 3 ppm or less, and glucose concentration to the range of 5g/l. or less as cell culture liquid, respectively is mentioned.
[0019] As a radius of gyration of the impeller in the above-mentioned culture apparatus, 1/4 or more [of a cultivation tank bore] and 3/8 or less range is mentioned. Moreover, as an area of one impeller, 1/15 or more magnitude of the lengthwise direction cross section of the cell culture liquid part of a cultivation tank is mentioned, and, specifically, 1/15 or more and 1/4 or less magnitude is mentioned. As number of sheets of an impeller, two or more number of sheets is mentioned, and, specifically, two or more sheets and four number of sheets or less are mentioned.
[0020] Moreover, eccentricity may be carried out so that a periphery part may be located [as opposed to / so that 0 degree - 15 degrees whenever / tilt-angle / of 5 degrees - about 15 degrees / may be made / as opposed to / so that, as for the impeller in the above-mentioned culture apparatus, cell culture liquid may tend to convect in the vertical direction / the revolving shaft / desirably and, as for an impeller, cell culture liquid may tend to flow in the centrifugal direction / the vertical line from a revolving shaft] more back than the inside. Furthermore, as die length of the lengthwise direction of an impeller, the about one to 1/4 die length of a rotation diameter is mentioned. Moreover, as a rotational speed of an impeller, the following rates are mentioned by 800cm/in the periphery part of an impeller, and, specifically, 300cm a part for /-, and 800cm rate for /are mentioned.
[0021]
[Function] In an approach according to claim 1 the cell culture liquid of an animal cell 1/4 or more [of the cultivation tank bore of the above / a radius of gyration] Since it agitates with a churning means to have the impeller formed in magnitude from which the area of 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] An animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.
[0022] In an approach according to claim 2, since rotational speed is made into the following by 800cm/in the periphery part of an impeller, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.
[0023] While an oxygen supply means to have a porous tube at least performs tube aeration and supplying oxygen into cell culture liquid in an approach according to claim 3 at an animal cell Since an animal cell is returned to a cultivation tank and new cell culture liquid is supplied to a cultivation tank with a culture medium supply means while carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank While being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.
[0024] In a configuration according to claim 4 1/4 or more [of the cultivation tank bore of the above / a radius of gyration] Since it has a churning means to have the impeller formed in magnitude from which the area of 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] and the above-mentioned cell culture liquid is agitated with this churning means It becomes possible [in large quantities] within a cultivation tank to cultivate to high density, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0025] In a configuration according to claim 5, since rotational speed is the following by 800cm/in the periphery part of an impeller, it becomes possible [in large quantities / cultivating to high density] within a cultivation tank, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0026] An oxygen supply means to have a porous tube at least, to perform tube aeration into cell culture liquid in a configuration according to claim 6, and to supply oxygen to an animal cell, Since it has a separation means to return an animal cell to a

cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank while carrying out centrifugal separation of cell culture liquid and the animal cell and sampling cell culture liquid out of a cultivation tank While being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.

[0027]

[Example] It will be as follows if one example of the culture apparatus of this invention is explained based on drawing 1 thru/or drawing 4 .

[0028] As shown in drawing 1 , the aeration tube (oxygen supply means) 3 and churning equipment (churning means) 4 are arranged on the cultivation tank 2 interior, and the culture-medium filling pipe (culture medium supply means) 5, the culture-medium sampling tubing (separation means) 6, the cell return tubing (separation means) 7, and an exhaust pipe 8 are arranged, and the culture apparatus 1 concerning this example is constituted. The above-mentioned culture apparatus 1 has the thermal resistance which is extent which can heat-sterilize.

[0029] The cultivation tank 2 is produced by hard glass, stainless steel, etc., and cell culture liquid (a culture medium is called hereafter) 10 is filled inside. Lid 2a is prepared in the cultivation tank 2, and the hole for inserting the aeration tube 3, the culture-medium filling pipe 5, the culture-medium sampling tubing 6, the cell return tubing 7, and an exhaust pipe 8 is established in this lid 2a. Moreover, the liquid level sensor, DO electrode (dissolved oxygen density measurement machine), and the pH electrode (neither is illustrated) are respectively attached in the cultivation tank 2 at the position. Furthermore, the outside is covered in the tank, the jacket (not shown), etc. and a cultivation tank 2 can perform now temperature control of the culture medium 10 in a cultivation tank 2 by adjusting the water temperature in a tank or a jacket.

[0030] Although a component is suitably chosen by the animal cell (a "cell" is only called the following which is not illustrated) cultivated, a culture medium 10 for example, the RDF-ITES culture medium (Murakami et al. —) which 2:1:1 came out of RPMI-1640 culture medium, the dull accretion strange method Eagle's medium, and F-hum 12 culture medium comparatively, and was mixed Agric.Biol.Chem., 46 (7), and 1831- 1937 and 1982 are made into a basal medium and the serum free medium changed by adding a minor constituent to this basal medium is used. Since the protein concentration in a culture medium is adjusted to 5 or less mg/l, in case the specified substance is separated from a culture medium, the bad influence by the protein component is reduced, and this serum free medium is tending to refine [come] the specified substance. Moreover, since there is little amount of the expensive protein used, the above-mentioned serum free medium is economical, when becoming cheap and performing continuous culture.

[0031] Although the optimal thing is chosen by the specified substance which produces the cell cultivated in a culture medium 10, when the specified substance is a Homo sapiens monoclonal antibody, lymphoid cells (Homo sapiens antibody forming cell), such as a recombination cell which introduced the Homo sapiens origin B cell, the mouse hybridoma, the Homo sapiens mouse hybridoma, and the antibody gene into the myeloma cell, are used, for example. The cell introduced into the culture medium 10 in a cultivation tank 2 makes the specified substance produce, after making it increase until it reaches cell density required for production of the specified substance.

[0032] Although the aeration tube 3 could pass neither the culture medium 10 nor the cell, for example, the countless hole of magnitude which can be passed carried out opening of oxygen or the carbon dioxide, it is produced by porous tubes, such as a porous fluororesin tube, a porous polypropylene tube, and a porous silicon tube, and it is formed spirally and immersed in the culture medium 10 in a cultivation tank 2. Although the optimal die length is determined by the diameter of inside and outside of the aeration tube 3, the class of cell to cultivate, the culture condition, etc., the die length of the aeration tube 3 For example, if it is the porous fluororesin tube (for example, pore chlorofluocarbon: the Sumitomo Electric Industries, Inc. make, Osaka) of the outer diameter of 3-10mm, the bore of 2-9mm, 50% of voidage, and the 2 micrometers of the maximum apertures, there should just be 0.3m or more per 1l. of culture media of die length of 1m or more preferably. Moreover, the oxygen-transfer coefficient (kLa) in this case is about 2-5l./hr that what is necessary is just to carry out aeration of air or the oxygen at the rate of 50 - 1000 ml/min. into the aeration tube 3.

[0033] While being pulled out by the cultivation tank 2 exterior by inserting point 3a of the aeration tube 3 in the hole established in lid 2a of a cultivation tank 2, end 3b of the aeration tube 3 is opened wide in the upper part of oil-level 10a of a culture medium 10. Moreover, point 3a of the aeration tube 3 is connected to a blower, an oxygen cylinder (neither is illustrated), etc. which carry out the supplied air of the air, and, thereby, aeration of air or the oxygen is carried out into the aeration tube 3. Therefore, while being able to carry out the gas exchange of the carbon dioxide in a culture medium 10, and the oxygen in the aeration tube 3 by tube aeration on aeration tube 3 front face, top-face aeration can also be performed by oil-level 10a of a culture medium 10. The air or oxygen emitted from end 3b of the aeration tube 3 is emitted to the cultivation tank 2 exterior by the exhaust pipe 8 inserted in the hole of lid 2a of a cultivation tank 2. Therefore, the oxygen consumption in the culture medium 10 by breathing of a cell can be compensated, growth of a cell can be urged, and the specified substance can be made to produce with the aeration tube 3.

[0034] Moreover, the cell density in a culture medium 10 is low at the time of culture initiation of a cell, and since the consumption of oxygen is small, the amount of supply of oxygen may also be small [the time]. Therefore, what is necessary is to carry out aeration of the air into the aeration tube 3, and just to keep dissolved oxygen concentration at 2-6 ppm. And if a logarithmic growth phase comes, it becomes high, and since the consumption of oxygen is large, cell density also needs to enlarge the amount of supply of oxygen. Therefore, what is necessary is to carry out aeration of the oxygen into the aeration tube 3, and just to control dissolved oxygen concentration to 0.1-3 ppm.

[0035] Furthermore, it connects also with the carbon-dioxide bomb (not shown), and point 3a of the aeration tube 3 can perform now pH adjustment of a culture medium 10 by carrying out aeration of the carbon dioxide into the aeration tube 3. In addition, by inserting end 3b of the aeration tube 3 in the hole established in lid 2a of a cultivation tank 2, without the ability opening wide in the upper part of oil-level 10a of a culture medium 10, it may pull out to the cultivation tank 2 exterior, and you may make it the configuration to which attach in instead the vent pipe which is not illustrated to lid 2a, supply air or oxygen independently [the aeration tube 3], and top-face aeration is made to carry out by oil-level 10a of a culture medium 10.

[0036] Churning equipment 4 is produced with stainless steel, a fluororesin, etc., by being inserted in the hole where revolving-shaft 4a was prepared in the core of cultivation tank 2 base, is pulled out by the cultivation tank 2 exterior and connected to the churning motor which is not illustrated. Impeller of at least two sheets 4b— is attached in the point of the above-mentioned revolving-shaft 4a. When the configuration of impeller 4b is formed in plate-like as shown in drawing 2 for example, although especially the configuration of impeller 4b is not limited in order to make a cell suspend in a culture medium 10 at the below-

mentioned rotational frequency, without doing damage to a cell — the length of a wing of impeller 4b — what is necessary is just to form in die length from which A (namely, radius of gyration of impeller 4b) becomes 1/4 or more [of the bore C of a cultivation tank 2], and 3/8 or less

[0037] The lengthwise direction cross section of the culture medium 10 when the area (AxB) of impeller 4b turns off a cultivation tank 2 to a lengthwise direction height B of impeller 4b 1/15 or more moreover, preferably although specifically based also on the depth of the culture medium 10 filled by the cultivation tank 2 that what is necessary is just to form in height which becomes 1/15 or more and 1/4 or less — height B — the length of a wing — what is necessary is just to form in height which becomes abbreviation 1 / two to twice to A Above-mentioned impeller 4b— makes 0-15 degrees whenever [tilt-angle / of about 5-15 degrees] preferably to revolving-shaft 4a, it is attached, and a culture medium 10 is tending to convect [come] in the vertical direction. In addition, to the vertical line from revolving-shaft 4a, eccentricity of impeller 4b— may be carried out, and it may be attached so that a periphery part may be located more back than the inside, so that a culture medium 10 may tend to flow in the centrifugal direction. Moreover, besides preparing perpendicularly to cultivation tank 2 base, to cultivation tank 2 base, revolving-shaft 4a may make the include angle of 20 degrees or less, and may prepare revolving-shaft 4a perpendicularly so that a culture medium 10 may tend to convect in the vertical direction.

[0038] Above-mentioned impeller 4b— is impeller 4b, in order to make a culture medium 10 suspend a cell, without doing damage to a cell. — The rotational speed of a periphery part below 800 cm/min. preferably It agitates more than 300 cm/min. at a rate which becomes below 800 cm/min. specifically Impeller 4b— agitates a culture medium 10 at the rotational frequency below 20r.p.m. more than 10r.p.m. preferably below 30r.p.m. more than 5r.p.m. here — temporary — the length of a wing of impeller 4b, if it forms in die length from which A becomes less than [of the bore C of a cultivation tank 2] 1/4 since a cell cannot be made to suspend in a culture medium 10 even if it rotates impeller 4b— at the rotational frequency of 30r.p.m. — not being desirable — moreover, the length of a wing of impeller 4b, if it forms in die length to which A becomes larger than three eighths of the bores C of a cultivation tank 2 Since a turbulent flow arises in a culture medium 10 between an impeller 4b wing tip and cultivation tank 2 wall and damage is done to a cell, it is not desirable. therefore — above — the length of a wing of impeller 4b — a cell can be made to suspend in a culture medium 10, without setting up A and doing damage to a cell by the thing of impeller 4b— for which a rotational frequency is controlled

[0039] In addition, revolving-shaft 4a of churning equipment 4 may be pulled out by the cultivation tank 2 exterior by being inserted in the hole established in lid 2a of a cultivation tank 2 instead of being pulled out by the cultivation tank 2 exterior by being inserted in the hole established in cultivation tank 2 base. In this case, since it is not necessary to establish a hole in cultivation tank 2 base, the structure of a cultivation tank 2 can be simplified.

[0040] As shown in drawing 1 , the above-mentioned culture-medium sampling tubing 6 and the above-mentioned cell return tubing 7 are produced with stainless steel, a fluororesin, etc., and are pulled out by the cultivation tank 2 exterior by being inserted in the hole established in lid 2a of a cultivation tank 2. And these tubing 6-7 is connected to the centrifugal separator (separation means) 11 through the pump which is not illustrated, and after separating the cell suspended in a culture medium 10, and the specified substance produced by the cell, the specified substance is collected while performing culture-medium exchange. That is, by extracting the supernatant liquid (supernatant) of a culture medium 10 with the culture-medium sampling tubing 6, carrying out centrifugal separation of a cell and the specified substance with a centrifugal separator 11, and sending the culture medium containing the specified substance to the specified substance cistern (separation means) 13 with the liquid-sending tubing 12, while returning the culture medium containing a cell near the pars basilaris ossis occipitalis of a cultivation tank 2 with the cell return tubing 7, the specified substance is collected at the same time it performs culture-medium exchange. In addition, the specified substance contained in the culture medium stored in the specified substance cistern 13 is isolated and refined by the predetermined approach.

[0041] The above-mentioned centrifugal separator 11 carries out centrifugal separation of the 300 or less xgs of the supernatant liquid of a culture medium 10 with the centrifugal acceleration of 100 or less xgs preferably, in order to separate a cell and the specified substance, without doing damage to a cell. In addition, the draw of the supernatant liquid of a culture medium 10 may be performed continuously, and may be performed intermittently. For example, although it is based on the capacity of a cultivation tank 2, the class of cell to cultivate in culture being in a steady state and performing the draw of the supernatant liquid of a culture medium 10 continuously, the centrifugal separator 11 should just have the capacity that the volume of 1 - 20 l/h can be processed by the aseptic condition (for example, cent RITEKKU CC 100: cent RITEKKU A BI, the product made from NOSUBORUGU, Sweden).

[0042] It connects with the culture-medium cistern (culture medium supply means) 14 containing a new culture medium, and the above-mentioned culture-medium filling pipe 5 is inserted into the cultivation tank 2 from the hole established in lid 2a of a cultivation tank 2. And the culture-medium filling pipe 5 supplies the culture medium containing the specified substance sent to the specified substance cistern 13, and a culture medium with new tales doses to a cultivation tank 2. In addition, supply of a new culture medium may be performed continuously and you may carry out intermittently. However, it is desirable to carry out continuously, if culture will be in a steady state.

[0043] The above-mentioned liquid level sensor (not shown) detects the location of oil-level 10a of the culture medium 10 in a cultivation tank 2, and inputs it into the control unit which does not illustrate this result. Similarly, a pH electrode (not shown) measures pH of a culture medium 10, and DO electrode (not shown) measures the dissolved oxygen concentration in a culture medium 10, and it inputs a result into a control unit respectively. This adjusts the amount of the oxygen which a control unit adjusts the amount of culture media extracted from the new amount of culture media supplied from the culture-medium filling pipe 5, or the culture-medium sampling tubing 6, or carries out aeration into the aeration tube 3, or a carbon dioxide. To 0.1 ppm or more and 3 ppm or less, glucose concentration is controlled to 0.1 or more g/l, lactic-acid concentration is controlled for the dissolved oxygen concentration in a culture medium 10 at 5 or less g/l, respectively, and the culture medium 10 in a cultivation tank 2 is always maintained at the optimal culture environment where growth of a cell and production of the specified substance are performed.

[0044] For example, since a possibility that a cell may receive damage by hyperoxia will arise if dissolved oxygen concentration increases more than 3 ppm on the other hand preferably, since a cell will be short of oxygen if the dissolved oxygen concentration in a culture medium 10 is set to less than 0.1 ppm, it is not desirable. Moreover, since the toxicity over a cell will become large if lactic-acid concentration increases more than 5 g/l on the other hand preferably since a cell will not fully increase if glucose concentration becomes inl. and less than 0.1g /, the activity of a cell falls and a survival rate falls, it is not desirable. In addition, what is necessary is just to carry out the rate of exchange of a culture medium 10 (perfusion ratio) in about 0.5 - 5 times/day to the amount of culture media in a cultivation tank 2, although based on a class, cell density, etc. of a cell to

cultivate.
[0045] Next, continuous culture of a cell is performed as one example of the culture approach of this invention using the culture apparatus 1 of the above-mentioned configuration, and the result measured for several days on the conditions which show below the cell density in a culture medium 10 and the concentration in the inside of the culture medium 10 of the specified substance produced by the cell is shown.
[0046] The S-RDF-ITES culture medium of a component presentation as shown in Table 1 which is a non-blood serum low protein culture medium which changed the RDF-ITES culture medium as one culture medium 10 was used for the above-mentioned conditions. The Homo sapiens transferrin (2.0 mg/l) and the cow insulin (2.0 mg/l) in Table 1 are a protein component, and, therefore, the protein concentration of a S-RDF-ITES culture medium is adjusted to 4.0 mg/l. Moreover, glucose concentration is adjusted to 5.0 g/l.
[0047]
[Table 1]

成分	濃度 (mg/l)
パラアミノ安息香酸	0.5
塩酸ピリドキシン	0.52
塩酸ピリドキサール	1.0
リボフラビン	0.21
塩酸チアミン	1.6
シアノコバラミン	0.3
リポ酸	0.05
リノール酸	0.02
塩化ナトリウム	6500
塩化カリウム	356
硫酸マグネシウム (無水)	48.8
塩化マグネシウム (無水)	14.3
塩化カルシウム (無水)	58.3
硝酸カルシウム (無水)	34.8
硫酸銅 (五水塩)	0.0006
硫酸亜鉛 (七水塩)	0.22
リン酸一水素ナトリウム (無水)	35.5
リン酸二水素ナトリウム (無水)	365.5
硝酸第二鉄 (九水塩)	0.025
硫酸第一鉄 (七水塩)	0.21
グルコース	4000
コハク酸ナトリウム (六水塩)	88.8
コハク酸	49.5
ビルビン酸ナトリウム	55.0
フェノールレッド	4.1
ヒトトランスフェリン	2.0
ウシインシュリン	2.0
亜セレン酸ナトリウム	0.004
エタノールアミン	1.5
2-メルカプトエタノール	0.0017
クエン酸ナトリウム	2.6
塩化第二鉄 (六水塩)	2.7

成分	濃度 (mg/l)
L-アルギニン塩酸塩	193.8
L-アラニン	2.2
L-アスパラギン (一水塩)	32.2
L-アスパラギン酸	13.3
L-システイン塩酸塩 (一水塩)	62.8
L-グルタミン酸	13.7
L-グルタミン	336.5
グリシン	14.4
L-ヒスチジン塩酸塩 (一水塩)	25.9
L-ヒドロキシプロリン	10.0
L-イソロイシン	52.2
L-ロイシン	54.5
L-リジン塩酸塩	65.7
L-メチオニン	16.1
L-フェニルアラニン	25.2
L-プロリン	18.6
L-セリン	28.1
L-スレオニン	36.8
L-トリプトファン	7.0
L-チロシン	29.2
L-バリン	36.3
グルタチオン	0.5
ビオチン	0.1
パントテン酸カルシウム	1.2
塩化コリン	6.0
葉酸	1.8
ヒポキサンチン	1.0
チミジン	0.18
i-イノシトール	23.8
プトレッシン二塩酸塩	0.04
ニコチン酸アミド	1.5

[0048] Moreover, the flat bottom jar fermenter (FZ2000: the product made from alpha RABARU, Sweden) with the bore of 250mm, a height [of 480mm], and a capacity of 20l. was used as a cultivation tank 2 of two culture apparatus 1. Temperature

control of the culture medium in a flat bottom jar fermenter was performed by adjusting the temperature of the water which it let pass in tubing in the jacket which has covered the jar-fermenter outside. 3) the length of a wing formed in plate-like as impeller 4b— whenever [tilt-angle / of 5 degrees] was made and attached to revolving-shaft 4a using the four-sheet aerofoil whose A is 80mm and whose height B is 160mm, and it was made to rotate at the rotational frequency of 5 - 15r.p.m. 4) As an aeration tube 3, with an outer diameter of 7mm porosity fluororesin tube (Biott make, Tokyo) 5m was rolled spirally, and it dedicated in the flat bottom jar fermenter. 5) The liquid level sensor (glass light sensor: made in the Fujiwara factory, Tokyo), the pH electrode (465-50-S7: the product made from in GORUTO, Switzerland), and DO electrode (phi25mm-70: the product made from in GORUTO, Switzerland) were attached in the above-mentioned flat bottom jar fermenter.

[0049] Furthermore, before culture of six cells performed main culture with the culture apparatus 1, it used the culture flask, 1l. round bottom flask, and 6l. round bottom flask, and performed preculture. That is, the cell which performs main culture with a culture apparatus 1 was proliferated by carrying out batch culture, after inoculating by predetermined cell density using a culture flask, 1l., and 6l. round bottom flask. Hereafter, the three above-mentioned kinds of flasks used for preculture are explained.

[0050] As a culture flask (not shown), the standing type tissue culture flask (T-flask: the Corning make, U.S.) with a capacity of 150ml was used.

[0051] As shown in drawing 3 , the glass spinner flask (the Shibata HARIO glass, Tokyo) 22 with a bore [of 100mm] and a height of 200mm was used as a 1l. round bottom flask. The length of a wing A1 formed in plate-like as impeller 24b— of the churning equipment 24 in this spinner flask 22 40mm and height B1 The two-sheet aerofoil which is 20mm was used. The aeration approach performed top-face aeration which permutes the gas in a flask. Moreover, the pH electrode (405-DPAS-K 8S/325: the product made from in GORUTO, Switzerland) which is not illustrated in the spinner flask 22 was attached. Furthermore, temperature control of the culture medium in the spinner flask 22 was performed by adjusting the water temperature of the tank (not shown) which has covered spinner flask 22 outside.

[0052] As shown in drawing 4 , the glass circular jar fermenter (the Shibata HARIO glass, Tokyo) 32 with a bore [of 190mm] and a height of 250mm was used as a 6l. round bottom flask. The length of a wing A2 formed in plate-like as impeller 34b— of the churning equipment 34 in this circular jar fermenter 32 40mm and height B-2 Whenever [tilt-angle / of 5 degrees] was made and attached to revolving-shaft 34a using the four-sheet aerofoil which is 80mm. As an aeration tube 33 which performs tube aeration, with an outer diameter of 7mm porosity fluororesin tube (Biott make, Tokyo) 3m was rolled spirally, and it dedicated in the circular jar fermenter 32. Moreover, pH electrode(405-DPAS-K 8S/325: product made from in GORUTO, Switzerland) 39a and DO electrode (phi14mm: B.E. MARUBISHI make, Tokyo) 39b were attached in the above-mentioned circular jar fermenter 32. Furthermore, temperature control of the culture medium in the circular jar fermenter 32 was performed by adjusting the water temperature of the tank (not shown) which has covered jar-fermenter 32 outside. In addition, culture environments, such as an agitating speed of churning equipment 34 and pH, were controlled using the control device (MCT-3S: the B.E. MARUBISHI make, Tokyo) which is not illustrated.

[0053] And under the conditions of above 1-6, the cell was suspended in predetermined cell density in the S-RDF-ITES culture medium (culture medium 10) in a flat bottom jar fermenter (cultivation tank 2), and main culture was started. Moreover, with growth of a cell, the culture-medium exchange or continuation perfusion using a centrifugal separator 11 was performed, and culture was continued. It is shown below by making a result into examples 1-3. However, the culture approach of this invention is not limited to examples 1-3.

[0054] [Example 1] The Homo sapiens Ig(immunoglobulin) M production Homo sapiens mouse hybridoma cell strain obtained by the cell fusion of mouse myeloma P3X63Ag8.653 and a Homo sapiens lymphocyte was used as a cell.

[0055] First, it is cell density 1.0x105 in 70ml of culture media in T-flask about a cell. If it inoculates and suspends so that it may be set to an individual/ml, and it puts at 37 degrees C under the ambient atmosphere of 5% of carbon dioxide levels, it will be cell density 7.4x105 after culture initiation on the 3rd. It increased to an individual/ml. Moreover, the antibody (specified substance, Homo sapiens IgM) concentration in the time of culture termination was 28 mg/l.

[0056] Next, it is cell density 1.0x105 in 500ml of culture media in the spinner flask 22 about the cell increased within T-flask. If it inoculates and suspends so that it may be set to an individual/ml, and it agitates at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 30r.p.m., it will be cell density 6.6x105 after culture initiation on the 3rd. It increased to an individual/ml. Moreover, the antibody concentration in the time of culture termination was 25 mg/l.

[0057] Furthermore, it is cell density 1.0x105 about the cell increased within the spinner flask 22 in 5l. of culture media in the circular jar fermenter 32. If it inoculates and suspends so that it may be set to an individual/ml, and it agitates at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 13r.p.m., it will be cell density 7.9x105 after culture initiation on the 3rd. It increased to an individual/ml. Moreover, the antibody concentration in the time of culture termination was 30 mg/l.

[0058] About the cell which carried out preculture as mentioned above, it is cell density 1.8x105 in 15l. of culture media in a flat bottom jar fermenter. It inoculated and suspended so that it might be set to an individual/ml, and main culture was agitated and carried out at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 10r.p.m. Moreover, the aeration rate in a porous fluororesin tube was made into 200-500ml/min., and the time of main culture initiation made the oxygen tension in air increase, and controlled dissolved oxygen concentration to 0.5-3 ppm as aeration of the air was carried out into the tube, dissolved oxygen concentration was kept at 2-6 ppm and the cell increased. Then, it will be cell density 6.8x105 after main culture initiation on the 3rd. It increased to an individual/ml. Perfusion was performed for 4 hours at this time, culture media were exchanged and perfusion was further performed continuously after after [main culture initiation] the 4th. In addition, the rate of exchange of a culture medium (perfusion ratio) was carried out in 1 - 1.3 times/day to the culture-medium volume (15l.) in a flat bottom jar fermenter. Moreover, the glucose concentration in a main culture period and a culture medium was controlled to 1 or more g/l, and lactic-acid concentration was controlled in 2.7g/l. or less, respectively.

[0059] If main culture of the cell is carried out for 15 days as mentioned above, it increases to cell density as shows in Table 2, and will be cell density 7.7x106 after main culture initiation on the 13th. It was set to an individual/ml. Moreover, the cell followed on increasing, as shown in Table 2, antibody concentration also increased, and antibody concentration became in l. and 105mg /after main culture initiation on the 12th.

[0060]

[Table 2]

培養日数 (日)	細胞密度 (個／ml)	抗体濃度 (mg／l)
1	1.8×10^5	—
3	6.8×10^5	—
4	7.4×10^5	3
5	1.4×10^6	8
6	2.0×10^6	30
7	2.3×10^6	41
8	3.1×10^6	52
9	3.7×10^6	62
10	5.1×10^6	75
11	5.9×10^6	69
12	6.8×10^6	105
13	7.7×10^6	76
14	7.5×10^6	82
15	7.5×10^6	72

[0061] The above result shows urging growth of a cell, and the productivity drive of the specified substance to the culture apparatus 1 and the culture approach of the above-mentioned configuration. That is, while it is possible to make it increase by the culture apparatus 1 and the culture approach of the above-mentioned configuration, without doing damage etc. to a cell, a cell is understood that it is extensive within a cultivation tank, and possible to cultivate to high density, and for it to be stabilized and to mass-produce efficiently the antibody which is the specified substance.
[0062] [Example 2] The mouse IgG production mouse hybridoma cell strain obtained by the cell fusion of mouse myeloma P3X63Ag8.653 and a mouse lymphocyte was used as a cell.
[0063] First, it is cell density 1.1x105 in 70ml of culture media in T-flask about a cell. If it inoculates and suspends so that it may be set to an individual/ml, and it puts at 37 degrees C under the ambient atmosphere of 5% of carbon dioxide levels, it will be cell density 1.15x106 after culture initiation on the 4th. It increased to an individual/ml. The doubling time of a cell was 28.1 hours.
[0064] Next, it is cell density 2.7x105 in 500ml of culture media in the spinner flask 22 about the cell increased within T-flask. If it inoculates and suspends so that it may be set to an individual/ml, and it agitates at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 20r.p.m., it will be cell density 8.2x105 after culture initiation on the 3rd. It increased to an individual/ml. The doubling time of a cell was 44.6 hours.
[0065] Furthermore, it is cell density 3.1x105 about the cell increased within the spinner flask 22 in 5l. of culture media in the circular jar fermenter 32. If it inoculates and suspends so that it may be set to an individual/ml, and it agitates at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 12r.p.m., it will be cell density 1.05x106 after culture initiation on the 2nd. It increased to an individual/ml. The doubling time of a cell was 27.0 hours.
[0066] About the cell which carried out preculture as mentioned above, it is cell density 2.9x105 in 14.5l. of culture media in a flat bottom jar fermenter. It inoculated and suspended so that it might be set to an individual/ml, and main culture was agitated and carried out at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 10r.p.m. Moreover, the aeration rate in a porous fluororesin tube was made into 200-500ml/min., and the time of main culture initiation made the oxygen tension in air increase, and controlled dissolved oxygen concentration to 0.5-3 ppm as aeration of the air was carried out into the tube, dissolved oxygen concentration was kept at 2-6 ppm and the cell increased. Then, it will be cell density 1.08x106 after main culture initiation on the 4th. It increased to an individual/ml. Perfusion was performed for 3 hours at this time, and culture media were exchanged. Moreover, it will be cell density 1.33x106 after main culture initiation on the 5th. It increased to an individual/ml. Perfusion was performed for 3 hours at this time, culture media were exchanged and perfusion was further performed continuously after after [main culture initiation] the 6th. In addition, the rate of exchange of a culture medium (perfusion ratio) was carried out in 1.5 - 2.0 times/day to the culture-medium volume (14.5l.) in a flat bottom jar fermenter. Moreover, the glucose concentration in a main culture period and a culture medium was controlled to 1.5 or more g/l, and lactic-acid concentration was controlled in 2.3g/l. or less, respectively.
[0067] If main culture of the cell is carried out for 18 days as mentioned above, it increases to cell density as shows in Table 3, and will be cell density 3.4x106 after main culture initiation on the 18th. It was set to an individual/ml.
[0068]
[Table 3]

培養日数 (日)	細胞密度 (個/ml)
1	2.9×10^{5}
4	1.1×10^{6}
5	1.3×10^{6}
6	1.5×10^{6}
7	1.5×10^{6}
8	1.8×10^{6}
9	1.7×10^{6}
10	1.8×10^{6}
11	1.7×10^{6}
12	1.8×10^{6}
13	2.0×10^{6}
14	2.2×10^{6}
15	2.3×10^{6}
16	2.7×10^{6}
17	2.7×10^{5}
18	3.4×10^{6}

[0069] The above result shows urging growth of a cell to the culture apparatus 1 and the culture approach of the above-mentioned configuration. That is, while it is possible to make it increase by the culture apparatus 1 and the culture approach of the above-mentioned configuration, without doing damage etc. to a cell, a cell is understood that cultivating to high density is extensive within a cultivation tank, and possible.
[0070] [Example 3] The Homo sapiens IgM production recombination cell which makes a Namalwa cell a host was used as a cell.
[0071] First, it is cell density 2.0x105 in 70ml of culture media in T-flask about a cell. If it inoculates and suspends so that it may be set to an individual/ml, and it puts at 37 degrees C under the ambient atmosphere of 5% of carbon dioxide levels, it will be cell density 9.4x105 after culture initiation on the 4th. It increased to an individual/ml. The doubling time of a cell was 43.7 hours. Moreover, the antibody (specified substance, Homo sapiens IgM) concentration in the time of culture termination was 5.8 mg/l.
[0072] Next, it is cell density 2.1x105 in 500ml of culture media in the spinner flask 22 about the cell increased within T-flask. If it inoculates and suspends so that it may be set to an individual/ml, and it agitates at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 15r.p.m., it will be cell density 1.27x106 after culture initiation on the 4th. It increased to an individual/ml. The doubling time of a cell was 33.8 hours. Moreover, the antibody concentration in the time of culture termination was 3 mg/l.
[0073] Furthermore, it is cell density 2.6x105 about the cell increased within the spinner flask 22 in 5l. of culture media in the circular jar fermenter 32. If it inoculates and suspends so that it may be set to an individual/ml, and it agitates at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 15r.p.m., it will be cell density 7.9x105 after culture initiation on the 5th. It increased to an individual/ml. The doubling time of a cell was 71.6 hours. Moreover, the antibody concentration in the time of culture termination was 3.9 mg/l.
[0074] About the cell which carried out preculture as mentioned above, it is cell density 2.3x105 in 15.8l. of culture media in a flat bottom jar fermenter. It inoculated and suspended so that it might be set to an individual/ml, and main culture was agitated and carried out at 37 degrees C, pH 6.8-7.1, and the rotational frequency of 10r.p.m. Moreover, the aeration rate in a porous fluororesin tube was made into 200-500ml/min., and the time of main culture initiation made the oxygen tension in air increase, and controlled dissolved oxygen concentration to 0.5-3 ppm as aeration of the air was carried out into the tube, dissolved oxygen concentration was kept at 2-6 ppm and the cell increased. Then, it increased to 9.2x105 cell density/ml after main culture initiation on the 3rd. Perfusion was performed for 3 hours at this time, and culture media were exchanged. It will be cell density 1.7x106 after main culture initiation on the 5th. It increased to an individual/ml. Perfusion was performed for 3 hours at this time, and culture media were exchanged. It increased to 2.7x106 cell density/ml after main culture initiation on the 6th. Perfusion was performed for 3 hours at this time, and culture media were exchanged. It will be cell density 4.1x106 after main culture initiation on the 7th. It increased to an individual/ml. Perfusion was performed for 3 hours at this time, culture media were exchanged and perfusion was further performed continuously after after [main culture initiation] the 8th. In addition, the rate of exchange of a culture medium (perfusion ratio) was carried out in 1.5 - 5 times/day to the culture-medium volume (15.8l.) in a flat bottom jar fermenter. Moreover, the glucose concentration in a main culture period and a culture medium was controlled to 1.5 or more g/l, and lactic-acid concentration was controlled in 2.5g/l. or less, respectively.
[0075] If main culture of the cell is carried out for 21 days as mentioned above, it increases to cell density as shows in Table 4, and will be cell density 1.8x107 after main culture initiation on the 20th. It was set to an individual/ml. Moreover, the cell followed on increasing, as shown in Table 4, antibody concentration also increased, and the antibody concentration on and after after [main culture initiation] the 11th was stabilized with 10 - 19 mg/l.
[0076]
[Table 4]

培養日数 (日)	細胞密度 (個／ml)	抗体濃度 (mg／l)
1	2.3×10^{5}	—
3	9.2×10^{5}	4
4	9.6×10^{5}	—
5	1.7×10^{6}	13
6	2.7×10^{6}	—
7	4.1×10^{6}	15
8	4.7×10^{6}	—
9	7.0×10^{6}	—
10	8.4×10^{6}	—
11	9.6×10^{6}	14
12	1.2×10^{7}	15
13	1.4×10^{7}	17
14	1.5×10^{7}	19
15	1.6×10^{7}	12
16	1.6×10^{7}	12
17	1.6×10^{7}	15
18	1.7×10^{7}	10
19	1.7×10^{7}	17
20	1.8×10^{7}	11
21	1.8×10^{7}	14

[0077] The above result shows urging growth of a cell, and the productivity drive of the specified substance to the culture apparatus 1 and the culture approach of the above-mentioned configuration. That is, while it is possible to make it increase by the culture apparatus 1 and the culture approach of the above-mentioned configuration, without doing damage etc. to a cell, a cell is understood that it is extensive within a cultivation tank, and possible to cultivate to high density, and for it to be stabilized and to mass-produce efficiently the antibody which is the specified substance.
[0078] In addition, the above-mentioned examples 1-3 show an example of a cell culture which used the culture approach of this invention, and the culture apparatus, and, of course, environments including culture, such as the dimension of cultivation tank 2 grade, the approach of preculture and equipment, a component presentation of a culture medium 10, a class of cell, and temperature, pH of a culture medium, etc. are not limit to a numeric value, a class, etc. which were showed in the above-mentioned examples 1-3, and they can change them suitably if needed. Moreover, of course, by the days shown in the above-mentioned examples 1-3, it is not closed and the culture days (period) of a cell can carry out [termination or] continuous culture of the cell over several months or several years using the culture approach of this invention, and a culture apparatus.
[0079]
[Effect of the Invention] The culture approach of the animal cell invention according to claim 1 is the approach of agitating the above-mentioned cell culture liquid with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] as mentioned above.
[0080] The effectiveness of an animal cell not receiving bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and becoming possible in large quantities within a cultivation tank to cultivate to high density by this is done so.
[0081] The culture approach of the animal cell invention according to claim 2 is the approach of making rotational speed the following by 800cm/in the periphery part of an impeller in the culture approach of an animal cell according to claim 1 as mentioned above.
[0082] The effectiveness of an animal cell not receiving bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and becoming possible in large quantities within a cultivation tank to cultivate to high density by this is done so.
[0083] The culture approach of the animal cell invention according to claim 3 As mentioned above, while an oxygen supply means to have a porous tube at least performs tube aeration and supplying oxygen into cell culture liquid at an animal cell in the culture approach of an animal cell according to claim 1 or 2 While carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank, it is the approach of returning an animal cell to a cultivation tank, and supplying new cell culture liquid to a cultivation tank with a culture medium supply means.
[0084] Thereby, while being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and the effectiveness of becoming possible in large quantities within a cultivation tank to cultivate to high density is done so.
[0085] The culture apparatus of the animal cell of invention according to claim 4 is a configuration which is equipped with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] as mentioned above, and agitates the above-mentioned cell culture liquid with this churning means.
[0086] This does so the effectiveness of becoming possible [in large quantities] within a cultivation tank to cultivate to high density, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.

[0087] In the culture apparatus of an animal cell according to claim 4, the rotational speed of the culture apparatus of the animal cell of invention according to claim 5 is the configuration of being the following by 800cm/, in the periphery part of an impeller as mentioned above.
[0088] This does so the effectiveness of becoming possible [in large quantities] within a cultivation tank to cultivate to high density, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0089] The culture apparatus of the animal cell of invention according to claim 6 As mentioned above, it sets to the culture apparatus of an animal cell according to claim 4 or 5. An oxygen supply means to have a porous tube at least, to perform tube aeration into cell culture liquid, and to supply oxygen to an animal cell, While carrying out centrifugal separation of cell culture liquid and the animal cell and sampling cell culture liquid out of a cultivation tank, it is a configuration equipped with a separation means to return an animal cell to a cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank.
[0090] Thereby, while being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and the effectiveness of becoming possible in large quantities within a cultivation tank to cultivate to high density is done so.

[Translation done.]

* NOTICES *

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TECHNICAL FIELD

[Industrial Application] By the continuous culture method, this invention relates to the culture approach of the animal cell which makes this animal cell produce the specified substance, such as a monoclonal antibody, and a culture apparatus while cultivating animal cells, such as an antibody forming cell.

[Translation done.]

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PRIOR ART

[Description of the Prior Art] Establishment of the mass culture approach of the animal cell in a industrial scale is becoming very important as a manufacture means for manufacturing biologicals, such as recent years, for example, interferon, erythropoietin, a colony stimulating factor, and a monoclonal antibody.
[0003] Although culture of an animal cell (a "cell" is only called hereafter) is performed the laboratory-scale which used culture containers, such as a culture flask and a spinner flask, in having scaled up such a small-scale culture approach simply in the industrial scale, it produces un-arranging in respiratory gas exchange, churning effectiveness, etc. of a cell, and cannot carry out mass culture of the cell. Then, the mass culture approach of a cell modeled after the mass culture approach using a microorganism is considered variously conventionally.
[0004] However, cells differ in respect of the following as compared with a microorganism. That is, since ** mechanical strength is weak, a cell tends to receive damage by churning, foaming, etc. of culture medium, as compared with a microorganism, in order to perform ** growth, it needs various nutrients and growth factors, and ** proliferation rate is very slow (doubling-time microorganism: during 0.2 - several hour, and cell: one - several days). For this reason, when mass culture of the cell is carried out using the jar fermenter (churning type culture apparatus) used for the mass culture of a microorganism, cell density cannot be made high in order that growth may stop, when a cell cannot fully grow in response to a mechanical damage etc. and the nutrient in a culture container is consumed. Therefore, when mass culture of the cell is carried out using the mass culture approach of a microorganism, it has the problem that the productive efficiency of the specified substance is very bad.
[0005] Then, in order to solve these problems, the continuous culture approach called high density perfusion cultivation is devised. By supplying the new culture medium containing a nutrient to a cultivation tank, high density perfusion cultivation maintains a cultivation tank at the optimal conditions for growth of a cell, and production of the specified substance, and is extensive and a method of cultivating to high density and producing the specified substance about a cell while it samples the supernatant liquid (supernatant) of cell culture liquid (a culture medium is called hereafter) including the specified substance and wastes out of a cultivation tank.
[0006] As the above-mentioned conventional high density perfusion cultivation, a cell is enclosed with the hollow filament cartridge which has the pore of microscopic smallness, for example. How (JP,62-171669,A) to proliferate a cell by exchanging nutriments etc. through the above-mentioned hollow filament, and to produce the specified substance, Fixed cultivation which fixes a cell to the front face of the support which consists of a ceramic etc., is made to increase a cell by exchanging nutriments etc. in the above-mentioned carrier surface, and produces the specified substance, such as an approach (OPUCHISERU (CHARU sliver, U.S.)), For example, although suspension culture, such as an approach (JP,62-134086,A) of making high density increasing a cell and producing the specified substance by combining the decollator which separates a jar fermenter, and an aforementioned cell and an aforementioned culture medium, is proposed Since the sampling of a cell, quality control of the specified substance, or enlargement of equipment is easy, promising ** of the suspension culture is carried out.

[Translation done.]

* NOTICES *

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EFFECT OF THE INVENTION

[Effect of the Invention] The culture approach of the animal cell invention according to claim 1 is the approach of agitating the above-mentioned cell culture liquid with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] as mentioned above.
[0080] The effectiveness of an animal cell not receiving bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and becoming possible in large quantities within a cultivation tank to cultivate to high density by this is done so.
[0081] The culture approach of the animal cell invention according to claim 2 is the approach of making rotational speed the following by 800cm/in the periphery part of an impeller in the culture approach of an animal cell according to claim 1 as mentioned above.
[0082] The effectiveness of an animal cell not receiving bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and becoming possible in large quantities within a cultivation tank to cultivate to high density by this is done so.
[0083] The culture approach of the animal cell invention according to claim 3 As mentioned above, while an oxygen supply means to have a porous tube at least performs tube aeration and supplying oxygen into cell culture liquid at an animal cell in the culture approach of an animal cell according to claim 1 or 2 While carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank, it is the approach of returning an animal cell to a cultivation tank, and supplying new cell culture liquid to a cultivation tank with a culture medium supply means.
[0084] Thereby, while being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and the effectiveness of becoming possible in large quantities within a cultivation tank to cultivate to high density is done so.
[0085] The culture apparatus of the animal cell of invention according to claim 4 is a configuration which is equipped with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] as mentioned above, and agitates the above-mentioned cell culture liquid with this churning means.
[0086] This does so the effectiveness of becoming possible [in large quantities] within a cultivation tank to cultivate to high density, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0087] In the culture apparatus of an animal cell according to claim 4, the rotational speed of the culture apparatus of the animal cell of invention according to claim 5 is the configuration of being the following by 800cm/, in the periphery part of an impeller as mentioned above.
[0088] This does so the effectiveness of becoming possible [in large quantities] within a cultivation tank to cultivate to high density, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0089] The culture apparatus of the animal cell of invention according to claim 6 As mentioned above, it sets to the culture apparatus of an animal cell according to claim 4 or 5. An oxygen supply means to have a porous tube at least, to perform tube aeration into cell culture liquid, and to supply oxygen to an animal cell, While carrying out centrifugal separation of cell culture liquid and the animal cell and sampling cell culture liquid out of a cultivation tank, it is a configuration equipped with a separation means to return an animal cell to a cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank.
[0090] Thereby, while being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and the effectiveness of becoming possible in large quantities within a cultivation tank to cultivate to high density is done so.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] However, in suspension culture, in order to maintain a cultivation tank at the optimal conditions for growth of a cell, and production of the specified substance, removal of the physical irritation (shearing force, foaming, etc. by churning of a culture medium) to a cell has been an important technical problem.
[0008] Since churning of a culture medium is performed by rotating the impeller formed in magnitude from which a radius of gyration becomes 1/4 or less [of a cultivation tank bore] at the rotational frequency more than 30r.p.m., a turbulent flow occurs in a culture medium, and it gives physical irritation, such as shearing force, to a cell, and has the problem of having bad influences, such as damage and destruction, on a cell.
[0009] Thus, in the above-mentioned conventional suspension culture, removal of the physical irritation (shearing force, foaming, etc. by churning of a culture medium) to a cell is inadequate. Therefore, it has come to perform mass culture of a cell.

[Translation done.]

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MEANS

[Means for Solving the Problem] Invention-in-this-application persons came to complete this invention, as a result of repeating examination wholeheartedly, in order to solve the above-mentioned technical problem.
[0011] Namely, the culture approach of the animal cell invention according to claim 1 In order to solve the above-mentioned technical problem, it is the culture approach of the animal cell cultivated in the cell culture liquid filled by the cultivation tank. It is characterized by agitating the above-mentioned cell culture liquid with a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank].
[0012] The culture approach of the animal cell invention according to claim 2 is characterized by making rotational speed into the following by 800cm/in the periphery part of an impeller in the culture approach of an animal cell according to claim 1, in order to solve the above-mentioned technical problem.
[0013] The culture approach of the animal cell invention according to claim 3 In order to solve the above-mentioned technical problem, while an oxygen supply means to have a porous tube at least performs tube aeration and supplying oxygen into cell culture liquid at an animal cell in the culture approach of an animal cell according to claim 1 or 2 While carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank, it is characterized by returning an animal cell to a cultivation tank, and supplying new cell culture liquid to a cultivation tank with a culture medium supply means.
[0014] The culture apparatus of the animal cell of invention according to claim 4 In order to solve the above-mentioned technical problem, it is the culture apparatus of the animal cell cultivated in the cell culture liquid filled by the cultivation tank. It has a churning means to have the impeller formed in magnitude from which the area whose radius of gyration is 1/4 or more [of the above-mentioned cultivation tank bore] and 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank]. It is characterized by agitating the above-mentioned cell culture liquid with this churning means.
[0015] In order that the culture apparatus of the animal cell of invention according to claim 5 may solve the above-mentioned technical problem, in the culture apparatus of an animal cell according to claim 4, rotational speed is characterized by 800cm being the following by /in the periphery part of an impeller.
[0016] The culture apparatus of the animal cell of invention according to claim 6 In order to solve the above-mentioned technical problem, it sets to the culture apparatus of an animal cell according to claim 4 or 5. An oxygen supply means to have a porous tube at least, to perform tube aeration into cell culture liquid, and to supply oxygen to an animal cell, While carrying out centrifugal separation of cell culture liquid and the animal cell and sampling cell culture liquid out of a cultivation tank, it is characterized by having a separation means to return an animal cell to a cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank.
[0017] As the above-mentioned culture approach, a suspended-cell-culture method etc. is mentioned, for example, as this suspended-cell-culture method, a batch culture method, a SEDDO batch culture method (a semibatch culture method, fed batch culture), a continuous culture method, etc. are mentioned, for example, and high density perfusion cultivation etc. is mentioned as the above-mentioned continuous culture method, for example.
[0018] Moreover, when performing a continuous culture method as the above-mentioned culture approach, there is the approach of dissociating using ** filter, ** centrifugal separation separating which is made to sediment with gravity using ** sedimentation tubing or a settling tank, and is separated among the separation approaches of a cell and a culture medium. Moreover, when using a Homo sapiens antibody forming cell as an animal cell, the serum-free culture liquid with which 0.1 or more g/l and lactic-acid concentration were adjusted [protein concentration / the dissolved oxygen concentration in 5 or less mg/l and cell culture liquid] for 0.1 ppm or more, 3 ppm or less, and glucose concentration to the range of 5g/l. or less as cell culture liquid, respectively is mentioned.
[0019] As a radius of gyration of the impeller in the above-mentioned culture apparatus, 1/4 or more [of a cultivation tank bore] and 3/8 or less range is mentioned. Moreover, as an area of one impeller, 1/15 or more magnitude of the lengthwise direction cross section of the cell culture liquid part of a cultivation tank is mentioned, and, specifically, 1/15 or more and 1/4 or less magnitude is mentioned. As number of sheets of an impeller, two or more number of sheets is mentioned, and, specifically, two or more sheets and four number of sheets or less are mentioned.
[0020] Moreover, eccentricity may be carried out so that a periphery part may be located [as opposed to / so that 0 degree - 15 degrees whenever / tilt-angle / of 5 degrees - about 15 degrees / may be made / as opposed to / so that, as for the impeller in the above-mentioned culture apparatus, cell culture liquid may tend to convect in the vertical direction / the revolving shaft / desirably and, as for an impeller, cell culture liquid may tend to flow in the centrifugal direction / the vertical line from a revolving shaft] more back than the inside. Furthermore, as die length of the lengthwise direction of an impeller, the about one to 1/4 die length of a rotation diameter is mentioned. Moreover, as a rotational speed of an impeller, the following rates are mentioned by 800cm/in the periphery part of an impeller, and, specifically, 300cm a part for /-, and 800cm rate for /are mentioned.

[Translation done.]

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OPERATION

[Function] In an approach according to claim 1 the cell culture liquid of an animal cell 1/4 or more [of the cultivation tank bore of the above / a radius of gyration] Since it agitates with a churning means to have the impeller formed in magnitude from which the area of 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] An animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.
[0022] In an approach according to claim 2, since rotational speed is made into the following by 800cm/in the periphery part of an impeller, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.
[0023] While an oxygen supply means to have a porous tube at least performs tube aeration and supplying oxygen into cell culture liquid in an approach according to claim 3 at an animal cell Since an animal cell is returned to a cultivation tank and new cell culture liquid is supplied to a cultivation tank with a culture medium supply means while carrying out centrifugal separation of cell culture liquid and the animal cell with a separation means and sampling cell culture liquid out of a cultivation tank While being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.
[0024] In a configuration according to claim 4 1/4 or more [of the cultivation tank bore of the above / a radius of gyration] Since it has a churning means to have the impeller formed in magnitude from which the area of 3/8 or less impeller [one] becomes 1/15 or more [of the lengthwise direction cross section of the cell culture liquid part of the above-mentioned cultivation tank] and the above-mentioned cell culture liquid is agitated with this churning means It becomes possible [in large quantities] within a cultivation tank to cultivate to high density, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0025] In a configuration according to claim 5, since rotational speed is the following by 800cm/in the periphery part of an impeller, it becomes possible [in large quantities / cultivating to high density] within a cultivation tank, without having bad influences by churning, such as damage, destruction, etc. by physical irritation, such as shearing force, on an animal cell.
[0026] An oxygen supply means to have a porous tube at least, to perform tube aeration into cell culture liquid in a configuration according to claim 6, and to supply oxygen to an animal cell, Since it has a separation means to return an animal cell to a cultivation tank, and a culture medium supply means to supply new cell culture liquid to a cultivation tank while carrying out centrifugal separation of cell culture liquid and the animal cell and sampling cell culture liquid out of a cultivation tank While being able to maintain the inside of a cultivation tank at the conditions optimal for an animal cell, an animal cell does not receive bad influences, such as damage and destruction, by physical irritation, such as shearing force by churning, and it becomes possible in large quantities within a cultivation tank to cultivate to high density.

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EXAMPLE

[Example] It will be as follows if one example of the culture apparatus of this invention is explained based on drawing 1 thru/or drawing 4 .
[0028] The culture apparatus 1 applied to this example as shown in drawing 1 is the aeration tube (oxygen supply means) 3 and churning equipment to the cultivation tank 2 interior.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the block diagram showing the configuration of the culture apparatus in one example of this invention.
[Drawing 2] It is the explanatory view showing the magnitude of the impeller in the above-mentioned culture apparatus.
[Drawing 3] It is the explanatory view of the spinner flask used for preculture.
[Drawing 4] It is the explanatory view of the circular jar fermenter used for preculture.
[Description of Notations]
1 Culture Apparatus
2 Cultivation Tank
3 Aeration Tube (Oxygen Supply Means)
4 Churning Equipment (Churning Means)
4b Impeller
5 Culture-Medium Filling Pipe (Culture Medium Supply Means)
6 Culture-Medium Sampling Tubing (Separation Means)
7 Cell Return Tubing (Separation Means)
10 Culture Medium (Cell Culture Liquid)
11 Centrifugal Separator (Separation Means)
13 Specified Substance Cistern (Separation Means)
14 Culture-Medium Cistern (Culture Medium Supply Means)
A Length of a wing (namely, radius of gyration)
B Height
C Bore

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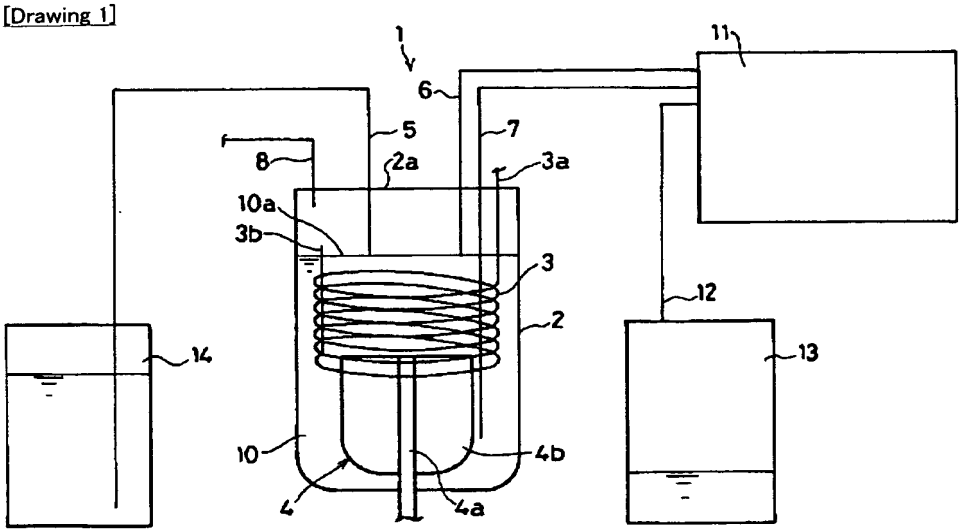
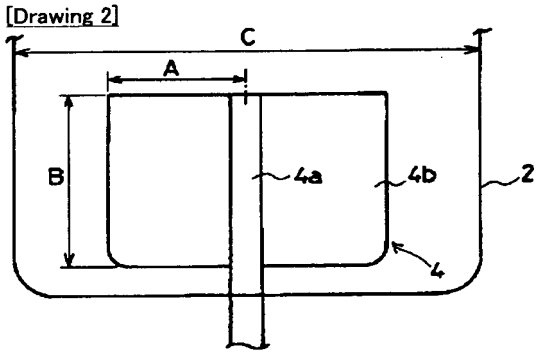
DRAWINGS

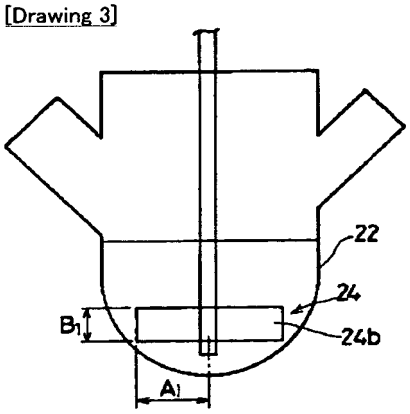
[Drawing 1]



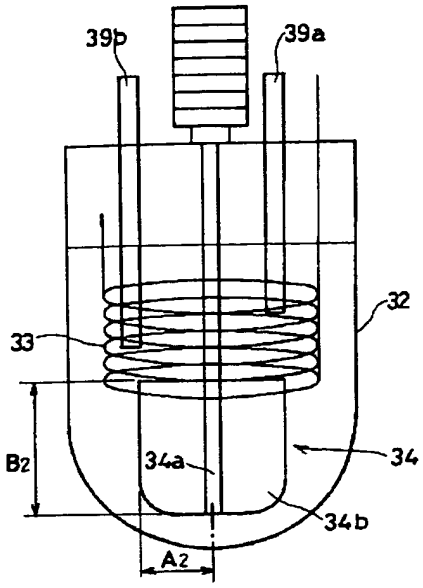
[Drawing 2]



[Drawing 3]



[Drawing 4]



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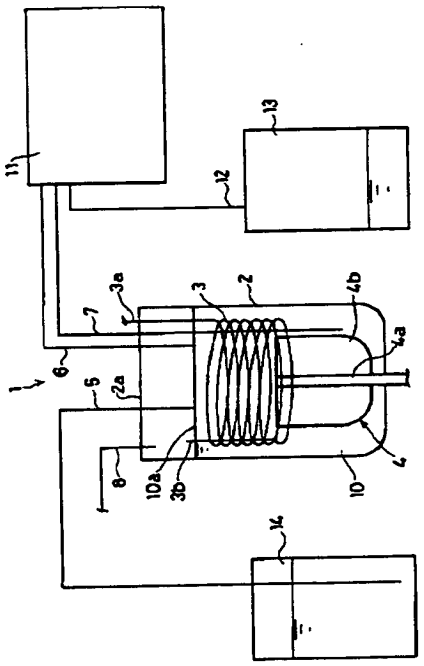
最終頁に続く

(54)【発明の名称】　動物細胞の培養方法および培養装置

(57)【要約】
【構成】　培養装置１は、培養槽２、細胞を培地１０中に懸濁させるための撹拌装置４、細胞に酸素を供給する通気チューブ３、培地１０と細胞とを分離する遠心分離機１１、および培養槽２に新しい培地を供給する培地貯液槽１４を備えている。撹拌装置４は、回転軸４ａに複数の撹拌翼４ｂ…を有し、これら撹拌翼４ｂ…は、回転半径が培養槽２内径の１／４以上、３／８以下となるような大きさに形成され、５r.p.m.以上、３０r.p.m.以下の回転数で回転される。
【効果】　細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが可能となる。



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【特許請求の範囲】

【請求項１】培養槽に満たされた細胞培養液中で培養する動物細胞の培養方法であって、

回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段で上記の細胞培養液を撹拌することを特徴とする動物細胞の培養方法。

【請求項２】回転速度を撹拌翼の外周部分で８００ｃｍ／分以下とすることを特徴とする請求項１記載の動物細胞の培養方法。

【請求項３】細胞培養液中に少なくとも多孔性チューブを有する酸素供給手段でチューブ通気を行って動物細胞に酸素を供給すると共に、分離手段で細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還し、かつ、培養液供給手段で培養槽に新しい細胞培養液を供給することを特徴とする請求項１または請求項２記載の動物細胞の培養方法。

【請求項４】培養槽に満たされた細胞培養液中で培養する動物細胞の培養装置であって、

回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段を備え、この撹拌手段により上記の細胞培養液を撹拌することを特徴とする動物細胞の培養装置。

【請求項５】回転速度が撹拌翼の外周部分で８００ｃｍ／分以下であることを特徴とする請求項４記載の動物細胞の培養装置。

【請求項６】少なくとも多孔性チューブを有し、細胞培養液中にチューブ通気を行って動物細胞に酸素を供給する酸素供給手段と、細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還する分離手段と、培養槽に新しい細胞培養液を供給する培養液供給手段とを備えていることを特徴とする請求項４または請求項５記載の動物細胞の培養装置。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、連続培養法により、例えば、抗体産生細胞等の動物細胞を培養すると共に、この動物細胞に例えばモノクローナル抗体等の目的物を生産させる動物細胞の培養方法および培養装置に関するものである。

【０００２】

【従来の技術】近年、例えば、インターフェロン、エリスロポエチン、コロニー刺激因子、モノクローナル抗体等の生物製剤を製造するための製造手段として、工業的規模での動物細胞の大量培養方法の確立が極めて重要となってきている。

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【０００３】動物細胞（以下、単に「細胞」と称する）の培養は、培養フラスコやスピナーフラスコ等の培養容器を用いた実験室的規模で行われているが、このような小規模の培養方法を工業的規模に単純にスケール・アップしたのでは、細胞の呼吸ガス交換や撹拌効率等に不都合を生じ、細胞を大量培養することができない。そこで、従来、微生物を用いた大量培養方法を参考にした細胞の大量培養方法が種々検討されている。

【０００４】ところが、細胞は微生物と比較して、以下の点で異なる。即ち、細胞は微生物と比較して、①機械的強度が弱いため、培養液の撹拌や発泡等により損傷を受け易く、②増殖を行うために多種の栄養素および増殖因子を必要とし、③増殖速度が極めて遅い（倍加時間微生物：０．２～数時間、細胞：１～数日間）。このため、微生物の大量培養に用いられるジャーファーメンター（撹拌式培養装置）等を用いて細胞を大量培養した場合、細胞が機械的損傷等を受けて充分に生育することができず、また、培養容器内の栄養分を消費した時点で増殖が停止するために細胞密度を高くすることができない。従って、微生物の大量培養方法を用いて細胞を大量培養した場合には、目的物の生産効率が非常に悪いという問題を有している。

【０００５】そこで、これらの問題を解消するために、高密度灌流培養法と称される連続培養方法が考案されている。高密度灌流培養法とは、目的物および老廃物を含んだ細胞培養液（以下、培地と称する）の上清（上澄み）を培養槽外へ抜き取ると共に、栄養素を含んだ新しい培地を培養槽へ供給することにより、培養槽を細胞の生育および目的物の生産に最適の条件に保ち、細胞を大量かつ高密度に培養して目的物を生産する方法である。

【０００６】上記従来の高密度灌流培養法としては、例えば、極微小の細孔を有する中空糸カートリッジに細胞を封入し、上記の中空糸を介して栄養物等を交換することで細胞を増殖させて目的物を生産する方法（特開昭６２－１７１６６９号公報）、セラミック等からなる担体の表面に細胞を固定し、上記の担体表面にて栄養物等を交換することで細胞を増殖させて目的物を生産する方法（オプチセル（チャールスリバー、米国））等の固定化培養法と、例えば、前記のジャーファーメンターと、細胞と培地とを分離する分離装置とを組み合わせることにより、細胞を高密度に増殖させて目的物を生産する方法（特開昭６２－１３４０８６号公報）等の懸濁培養法とが提案されているが、細胞のサンプリングや目的物の品質管理、あるいは装置の大型化が容易なことから、懸濁培養法が有望視されている。

【０００７】

【発明が解決しようとする課題】しかしながら、懸濁培養法において、培養槽を細胞の生育および目的物の生産に最適の条件に保つには、細胞に対する物理的刺激（培

地の撹拌による剪断力や発泡等）の除去が重要な課題となっている。

【０００８】培地の撹拌は、例えば、回転半径が培養槽内径の１／４以下となるような大きさに形成された撹拌翼を３０r.p.m.以上の回転数で回転して行われているため、培地に乱流が発生して細胞に剪断力等の物理的刺激を与えて、細胞に損傷や破壊等の悪影響を及ぼすという問題を有している。

【０００９】このように、上記従来の懸濁培養法においては、細胞に対する物理的刺激（培地の撹拌による剪断力や発泡等）の除去が不十分となっている。従って、細胞の大量培養を行うには到っていない。

【００１０】

【課題を解決するための手段】本願発明者らは上記課題を解決するために鋭意検討を重ねた結果、本発明を完成させるに至った。

【００１１】即ち、請求項１記載の発明の動物細胞の培養方法は、上記の課題を解決するために、培養槽に満たされた細胞培養液中で培養する動物細胞の培養方法であって、回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段で上記の細胞培養液を撹拌することを特徴としている。

【００１２】請求項２記載の発明の動物細胞の培養方法は、上記の課題を解決するために、請求項１記載の動物細胞の培養方法において、回転速度を撹拌翼の外周部分で８００ｃｍ／分以下とすることを特徴としている。

【００１３】請求項３記載の発明の動物細胞の培養方法は、上記の課題を解決するために、請求項１または請求項２記載の動物細胞の培養方法において、細胞培養液中に少なくとも多孔性チューブを有する酸素供給手段でチューブ通気を行って動物細胞に酸素を供給すると共に、分離手段で細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還し、かつ、培養液供給手段で培養槽に新しい細胞培養液を供給することを特徴としている。

【００１４】請求項４記載の発明の動物細胞の培養装置は、上記の課題を解決するために、培養槽に満たされた細胞培養液中で培養する動物細胞の培養装置であって、回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段を備え、この撹拌手段により上記の細胞培養液を撹拌することを特徴としている。

【００１５】請求項５記載の発明の動物細胞の培養装置は、上記の課題を解決するために、請求項４記載の動物細胞の培養装置において、回転速度が撹拌翼の外周部分で８００ｃｍ／分以下であることを特徴としている。

【００１６】請求項６記載の発明の動物細胞の培養装置は、上記の課題を解決するために、請求項４または請求項５記載の動物細胞の培養装置において、少なくとも多孔性チューブを有し、細胞培養液中にチューブ通気を行って動物細胞に酸素を供給する酸素供給手段と、細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還する分離手段と、培養槽に新しい細胞培養液を供給する培養液供給手段とを備えていることを特徴としている。

【００１７】上記の培養方法としては、例えば浮遊培養法等が挙げられ、この浮遊培養法としては、例えばバッチ培養法、セッドバッチ培養法（半回分培養法、流加培養法）、連続培養法等が挙げられ、上記の連続培養法としては、例えば高密度灌流培養法等が挙げられる。

【００１８】また、上記の培養方法として連続培養法を行う場合、細胞と培地との分離方法には、①沈降管もしくは沈降槽を用いて重力により沈降させて分離する、②フィルターを用いて分離する、③遠心分離により分離する等の方法がある。また、動物細胞としてヒト抗体産生細胞を用いる場合、細胞培養液としては蛋白濃度が５ｍｇ／ｌ以下、細胞培養液中の溶存酸素濃度が０．１ｐｐｍ以上、３ｐｐｍ以下、グルコース濃度が０．１ｇ／ｌ以上、乳酸濃度が５ｇ／ｌ以下の範囲にそれぞれ調整された無血清培養液が挙げられる。

【００１９】上記の培養装置における撹拌翼の回転半径としては培養槽内径の１／４以上、３／８以下の範囲が挙げられる。また、撹拌翼１枚の面積としては培養槽の細胞培養液部の縦方向断面積の１／１５以上の大きさが挙げられ、具体的には、１／１５以上、１／４以下の大きさが挙げられる。撹拌翼の枚数としては２枚以上の枚数が挙げられ、具体的には、２枚以上、４枚以下の枚数が挙げられる。

【００２０】また、上記の培養装置における撹拌翼は、細胞培養液が上下方向に対流し易いように、回転軸に対して０°～１５°、望ましくは５°～１５°程度の傾斜角度をなしていてもよく、また、撹拌翼は、細胞培養液が遠心方向に流れ易いように、回転軸からの垂直線に対して外周部分が内側よりも後方に位置するよう偏心していてもよい。さらに、撹拌翼の縦方向の長さとしては回転直径の１～１／４程度の長さが挙げられる。その上、撹拌翼の回転速度としては撹拌翼の外周部分で８００ｃｍ／分以下の速度が挙げられ、具体的には、３００ｃｍ／分～８００ｃｍ／分の速度が挙げられる。

【００２１】

【作用】請求項１記載の方法においては、動物細胞の細胞培養液を、回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段で撹拌するので、動物細胞が撹拌による剪断力等の物理的刺激に

より損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが可能となる。

【００２２】請求項２記載の方法においては、回転速度を撹拌翼の外周部分で８００ｃｍ／分以下とするので、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内でより大量かつ高密度に培養することが可能となる。

【００２３】請求項３記載の方法においては、細胞培養液中に少なくとも多孔性チューブを有する酸素供給手段でチューブ通気を行って動物細胞に酸素を供給すると共に、分離手段で細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還し、かつ、培養液供給手段で培養槽に新しい細胞培養液を供給するので、培養槽内を動物細胞にとって最適の条件に保つことができると共に、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが可能となる。

【００２４】請求項４記載の構成においては、回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段を備え、この撹拌手段により上記の細胞培養液を撹拌するので、動物細胞に、撹拌による剪断力等の物理的刺激による損傷や破壊等の悪影響を与えずに、培養槽内で大量かつ高密度に培養することが可能となる。

【００２５】請求項５記載の構成においては、回転速度が撹拌翼の外周部分で８００ｃｍ／分以下であるので、動物細胞に、撹拌による剪断力等の物理的刺激による損傷や破壊等の悪影響を与えずに、培養槽内でより大量かつ高密度に培養することが可能となる。

【００２６】請求項６記載の構成においては、少なくとも多孔性チューブを有し、細胞培養液中にチューブ通気を行って動物細胞に酸素を供給する酸素供給手段と、細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還する分離手段と、培養槽に新しい細胞培養液を供給する培養液供給手段とを備えているので、培養槽内を動物細胞にとって最適の条件に保つことができると共に、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが可能となる。

【００２７】

【実施例】本発明の培養装置の一実施例について図１ないし図４に基づいて説明すれば、以下の通りである。

【００２８】図１に示すように、本実施例にかかる培養装置１は、培養槽２内部に、通気チューブ（酸素供給手段）３と撹拌装置（撹拌手段）４とが配されており、また、培地注入管（培養液供給手段）５、培地抜き取り管（分離手段）６、細胞返却管（分離手段）７、および排気管８が配されて構成されている。上記の培養装置１は、加熱滅菌が行える程度の耐熱性を有している。

【００２９】培養槽２は、例えば、硬質ガラスやステンレス等で作製されており、内部には細胞培養液（以下、培地と称する）１０が満たされている。培養槽２には蓋２ａが設けられており、この蓋２ａには、通気チューブ３、培地注入管５、培地抜き取り管６、細胞返却管７、および排気管８を挿入するための穴が設けられている。また、培養槽２には、液面センサ、ＤＯ電極（溶存酸素濃度測定器）、およびｐＨ電極（何れも図示せず）が各々所定の位置に取り付けられている。さらに、培養槽２は、水槽やジャケット（図示せず）等で外側が覆われており、水槽やジャケット内の水温を調節することで培養槽２内の培地１０の温度制御を行うことができるようになっている。

【００３０】培地１０は、培養される動物細胞（図示しない、以下、単に「細胞」と称する）により適宜成分が選択されるが、例えば、ＲＰＭＩ－１６４０培地、ダルベコ変法イーグル培地およびハムＦ－１２培地を２：１：１の割合で混合したＲＤＦ－ＩＴＥＳ培地（村上ら、Ａｇｒｉｃ．Ｂｉｏｌ．Ｃｈｅｍ．，４６（７），１８３１－１９３７，１９８２）を基礎培地とし、この基礎培地に微量成分を添加することにより改変した無血清培地が用いられる。この無血清培地は、培地中の蛋白濃度が５ｍｇ／ｌ以下に調整されているので、培地から目的物を分離する際に蛋白成分による悪影響が低減され、目的物を精製し易いようになっている。また、高価な蛋白の使用量が少ないため、上記の無血清培地は安価となり、連続培養を行う上で経済的となっている。

【００３１】培地１０中で培養される細胞は、生産する目的物により最適のものが選択されるが、例えば、目的物がヒトモノクローナル抗体である場合には、ヒト由来Ｂ細胞、マウスハイブリドーマ、ヒト・マウスハイブリドーマ、抗体遺伝子をミエローマ細胞に導入した組み換え細胞等のリンパ系細胞（ヒト抗体産生細胞）が用いられる。培養槽２内の培地１０に導入された細胞は、目的物の生産に必要な細胞密度に達するまで増殖させた後、目的物を生産させるようになっている。

【００３２】通気チューブ３は、培地１０や細胞は通過できないが、酸素や二酸化炭素は通過できる大きさの無数の孔が開口した、例えば、多孔性フッ素樹脂チューブ、多孔性ポリプロピレンチューブ、多孔性シリコンチューブ等の多孔性チューブで作製されており、螺旋状に形成されて培養槽２内の培地１０に浸漬されている。通気チューブ３の長さは、通気チューブ３の内外径、培養する細胞の種類、培養条件等により最適の長さが決定されるが、例えば、外径３～１０ｍｍ、内径２～９ｍｍ、空隙率５０％、最大孔径２μｍの多孔性フッ素樹脂チューブ（例えば、ポアフロン：住友電工株式会社製、大

阪）であれば、培地１１当たり０．３m以上、好ましくは１m以上の長さがあればよい。また、通気チューブ３内には、空気または酸素を５０～１０００ｍｌ／ｍｉｎ．の速度で通気すればよく、この場合の酸素移動容量係数（ｋＬａ）は、約２～５１／ｈｒである。

【００３３】通気チューブ３の先端部３ａは、培養槽２の蓋２ａに設けられた穴に挿入されることにより培養槽２外部に引き出される一方、通気チューブ３の末端部３ｂは、培地１０の液面１０ａの上方で開放されている。また、通気チューブ３の先端部３ａは、空気を送気する送風機や酸素ボンベ（何れも図示せず）等に接続されており、これにより、通気チューブ３内に空気または酸素が通気されている。よって、通気チューブ３表面で、培地１０中の二酸化炭素と通気チューブ３内の酸素とをチューブ通気によりガス交換することができると共に、培地１０の液面１０ａで上面通気も行えるようになっている。通気チューブ３の末端部３ｂから放出された空気または酸素は、培養槽２の蓋２ａの穴に挿入された排気管８により、培養槽２外部に放出される。従って、通気チューブ３により、細胞の呼吸による培地１０中の酸素消費を補って細胞の増殖を促し、目的物を生産させることができる。

【００３４】また、細胞の培養開始時は培地１０中の細胞密度が低く、酸素の消費量が小さいため、酸素の供給量も小さくてよい。従って、通気チューブ３内に空気を通気して溶存酸素濃度を２～６ｐｐｍに保つようにすればよい。そして、対数増殖期になると細胞密度が高くなり、酸素の消費量が大きいため、酸素の供給量も大きくする必要がある。従って、通気チューブ３内に酸素を通気して溶存酸素濃度を０．１～３ｐｐｍに制御すればよい。

【００３５】さらに、通気チューブ３の先端部３ａは、二酸化炭素ボンベ（図示せず）にも接続されており、通気チューブ３内に二酸化炭素を通気することにより、培地１０のｐＨ調整を行うことができるようになっている。尚、通気チューブ３の末端部３ｂを培地１０の液面１０ａの上方で開放せずに培養槽２の蓋２ａに設けられた穴に挿入することにより培養槽２外部に引き出し、代わりに、蓋２ａに図示しない通気管を取り付けて通気チューブ３とは別に空気または酸素を供給して培地１０の液面１０ａで上面通気を行わせる構成にしてもよい。

【００３６】撹拌装置４は、例えば、ステンレスやフッ素樹脂等で作製されており、回転軸４ａが培養槽２底面の中心に設けられた穴に挿入されることにより培養槽２外部に引き出されて、図示しない撹拌モータに接続されている。上記の回転軸４ａの先端部には、少なくとも２枚の撹拌翼４ｂ…が取り付けられている。撹拌翼４ｂの形状は特に限定されないが、図２に示すように、例えば、撹拌翼４ｂの形状を平板状に形成した場合には、細胞に損傷を与えることなく、後述の回転数で細胞を培地１０中に懸濁させるために、撹拌翼４ｂの翼長Ａ（即ち、撹拌翼４ｂの回転半径）が培養槽２の内径Ｃの１／４以上、３／８以下となるような長さに形成すればよい。

【００３７】また、撹拌翼４ｂの高さＢは、撹拌翼４ｂの面積（Ａ×Ｂ）が、培養槽２を縦方向に切った場合における培地１０の縦方向断面積の１／１５以上、好ましくは、１／１５以上、１／４以下となるような高さに形成すればよく、具体的には、培養槽２に満たされる培地１０の液深にもよるが、高さＢが翼長Ａに対して略１／２～２倍となるような高さに形成すればよい。上記の撹拌翼４ｂ…は、回転軸４ａに対して、０～１５°、好ましくは、５～１５°程度の傾斜角度をなして取り付けられており、培地１０が上下方向に対流し易いようになっている。尚、撹拌翼４ｂ…は、培地１０が遠心方向に流れ易いように、回転軸４ａからの垂直線に対して、外周部分が内側より後方に位置するように偏心させて取り付けてもよい。また、回転軸４ａは、培養槽２底面に対して垂直方向に設ける以外に、培地１０が上下方向に対流し易いように、回転軸４ａを培養槽２底面に対して垂直方向から２０°以下の角度をなして設けてもよい。

【００３８】上記の撹拌翼４ｂ…は、細胞に損傷を与えることなく、細胞を培地１０に懸濁させるために、撹拌翼４ｂ…の外周部分の回転速度が８００ｃｍ／ｍｉｎ．以下、好ましくは、３００ｃｍ／ｍｉｎ．以上、８００ｃｍ／ｍｉｎ．以下となるような速度で撹拌するようになっており、具体的には、撹拌翼４ｂ…は、培地１０を５r.p.m.以上、３０r.p.m.以下、好ましくは、１０r.p.m.以上、２０r.p.m.以下の回転数で撹拌するようになっている。ここで、仮に、撹拌翼４ｂの翼長Ａが培養槽２の内径Ｃの１／４未満となるような長さに形成すると、撹拌翼４ｂ…を３０r.p.m.の回転数で回転させても細胞を培地１０中に懸濁させることができないので好ましくなく、また、撹拌翼４ｂの翼長Ａが培養槽２の内径Ｃの３／８よりも大きくなるような長さに形成すると、撹拌翼４ｂ翼端と培養槽２内壁との間で培地１０に乱流が生じて細胞に損傷を与えるので好ましくない。従って、上記のように撹拌翼４ｂの翼長Ａを設定し、かつ、撹拌翼４ｂ…の回転数を制御することで、細胞に損傷を与えることなく、細胞を培地１０中に懸濁させることができるようになっている。

【００３９】尚、撹拌装置４の回転軸４ａは、培養槽２底面に設けられた穴に挿入されることで培養槽２外部に引き出される代わりに、培養槽２の蓋２ａに設けられた穴に挿入されることで培養槽２外部に引き出されてもよい。この場合は、培養槽２底面に穴を設ける必要がないので、培養槽２の構造を簡略化することができる。

【００４０】図１に示すように、上記の培地抜き取り管６および細胞返却管７は、例えば、ステンレスやフッ素樹脂等で作製されており、培養槽２の蓋２ａに設けられ

た穴に挿入されることにより培養槽２外部に引き出されている。そして、これら管６・７は、図示しないポンプ等を介して遠心分離機（分離手段）１１に接続されており、培地１０中に懸濁している細胞と、細胞によって生産された目的物とを分離した後、培地交換を行うと共に目的物を回収するようになっている。即ち、培地１０の上清（上澄み）を培地抜き取り管６で抜き出して、遠心分離機１１で細胞と目的物とを遠心分離し、細胞を含んだ培地を細胞返却管７で培養槽２の底部近傍に戻すと共に、目的物を含んだ培地を送液管１２で目的物貯液槽（分離手段）１３に送ることにより、培地交換を行うと同時に目的物を回収している。尚、目的物貯液槽１３に貯えられた培地に含まれる目的物は、所定の方法により単離、精製される。

【００４１】上記の遠心分離機１１は、細胞に損傷を与えることなく、細胞と目的物とを分離するために、培地１０の上清を３００ｘｇ以下、好ましくは、１００ｘｇ以下の遠心加速度で遠心分離するようになっている。尚、培地１０の上清の抜き出しは、連続的に行ってもよく、間歇的に行ってもよい。例えば、培養が定常状態となり、培地１０の上清の抜き出しを連続的に行う場合には、培養槽２の容量や培養する細胞の種類等にもよるが、遠心分離機１１は１～２０１／ｈの液量を無菌状態で処理できる能力を有していればよい（例えば、セントリテックＣＣ１００：セントリテック・エー・ビー、ノースボルグ製、スウェーデン）。

【００４２】上記の培地注入管５は、新しい培地が入っている培地貯液槽（培養液供給手段）１４に接続されており、培養槽２の蓋２ａに設けられた穴から培養槽２内に挿入されている。そして、培地注入管５は、目的物貯液槽１３に送られる目的物を含んだ培地と同量の新しい培地を、培養槽２に供給するようになっている。尚、新しい培地の供給は、連続的に行ってもよく、間歇的に行ってもよい。但し、培養が定常状態になれば連続的に行うことが望ましい。

【００４３】上記の液面センサ（図示せず）は、培養槽２内における培地１０の液面１０ａの位置を検知して、この結果を、図示しない制御装置に入力する。同様に、ｐＨ電極（図示せず）は、培地１０のｐＨを測定し、また、ＤＯ電極（図示せず）は、培地１０中の溶存酸素濃度を測定して、各々結果を制御装置に入力する。これにより、制御装置は、培地注入管５から供給される新しい培地量や培地抜き取り管６から抜き出される培地量を調節し、あるいは通気チューブ３内に通気する酸素や二酸化炭素の量を調節して、培地１０中の溶存酸素濃度を０．１ｐｐｍ以上、３ｐｐｍ以下に、グルコース濃度を０．１ｇ／ｌ以上に、乳酸濃度を５ｇ／ｌ以下にそれぞれ制御し、常に、培養槽２内の培地１０を細胞の増殖や目的物の生産を行う最適の培養環境に保つようになっている。

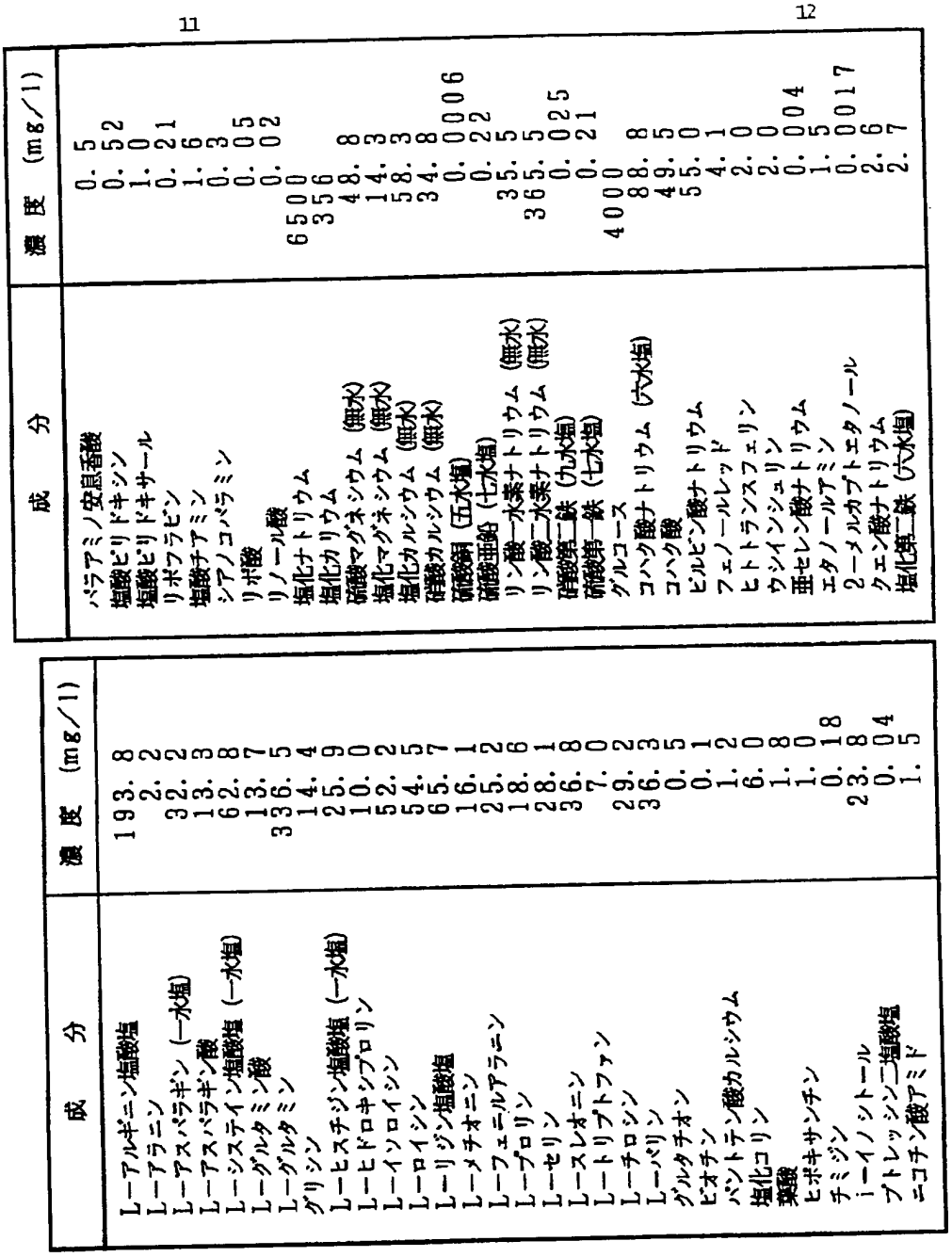
【００４４】例えば、培地１０中の溶存酸素濃度が０．１ｐｐｍ未満となると細胞が酸欠状態となるので好ましくなく、一方、溶存酸素濃度が３ｐｐｍより多くなると細胞が酸素過多により損傷を受ける虞れが生じるので好ましくない。また、グルコース濃度が０．１ｇ／ｌ未満となると細胞が充分に増殖しないので好ましくなく、一方、乳酸濃度が５ｇ／ｌより多くなると細胞に対する毒性が大きくなり、細胞の活性が低下して生存率が低下するので好ましくない。尚、培地１０の交換率（潅流比）は、培養する細胞の種類や細胞密度等にもよるが、培養槽２内の培地量に対し、０．５～５回／日程度とすればよい。

【００４５】次に、本発明の培養方法の一実施例として、上記構成の培養装置１を用いて細胞の連続培養を行い、培地１０中の細胞密度、および細胞により生産された目的物の培地１０中での濃度を、以下に示す条件で数日間測定した結果を示す。

【００４６】上記の条件は、１）培地１０として、ＲＤＦ－ＩＴＥＳ培地を改変した無血清低蛋白培地である、表１に示すような成分組成のＳ－ＲＤＦ－ＩＴＥＳ培地を用いた。表１中におけるヒトトランスフェリン（２．０ｍｇ／ｌ）およびウシインシュリン（２．０ｍｇ／ｌ）が蛋白成分であり、よって、Ｓ－ＲＤＦ－ＩＴＥＳ培地の蛋白濃度は４．０ｍｇ／ｌに調整されている。また、グルコース濃度は５．０ｇ／ｌに調整されている。

【００４７】

【表１】



成分	濃度 (mg/l)
パラアミノ安息香酸	0.5
塩酸ピリドキシン	0.52
塩酸ピリドキサール	1.0
リボフラビン	0.21
塩酸チアミン	1.6
シアノコバラミン	0.3
リボ酸	0.05
リノール酸	0.02
塩化ナトリウム	6500
塩化カリウム	356
硫酸マグネシウム (無水)	48.8
塩化マグネシウム (無水)	14.3
塩化カルシウム (無水)	58.3
硝酸カルシウム (無水)	34.8
硫酸銅 (五水塩)	0.0006
硫酸亜鉛 (七水塩)	0.22
リン酸一水素ナトリウム (無水)	35.5
リン酸二水素ナトリウム (無水)	365.5
硝酸第二鉄 (九水塩)	0.025
硫酸第一鉄 (七水塩)	0.21
グルコース	4000
コハク酸ナトリウム (六水塩)	88.8
コハク酸	49.5
ビルビン酸ナトリウム	55.0
フェノールレッド	4.1
ヒトトランスフェリン	2.0
ウシインシュリン	2.0
亜セレン酸ナトリウム	0.004
エタノールアミン	1.5
2－メルカプトエタノール	0.0017
クエン酸ナトリウム	2.6
塩化第二鉄 (六水塩)	2.7

成分	濃度 (mg/l)
L－アルギニン塩酸塩	193.8
L－アラニン	22.2
L－アスパラギン (一水塩)	32.3
L－アスパラギン酸	13.3
L－システイン塩酸塩 (一水塩)	62.8
L－グルタミン酸	13.7
L－グルタミン	336.5
グリシン	14.4
L－ヒスチジン塩酸塩 (一水塩)	25.9
L－ヒドロキシプロリン	10.0
L－イソロイシン	52.2
L－ロイシン	54.5
L－リジン塩酸塩	65.7
L－メチオニン	16.1
L－フェニルアラニン	25.2
L－プロリン	18.6
L－セリン	28.1
L－スレオニン	36.8
L－トリプトファン	7.0
L－チロシン	29.2
L－バリン	36.3
グルタチオン	0.5
ビオチン	0.12
パントテン酸カルシウム	1.2
塩化コリン	6.0
葉酸	1.8
ヒポキサンチン	1.0
チミジン	0.18
i－イノシトール	23.8
プトレッシン二塩酸塩	0.04
ニコチン酸アミド	1.5

【００４８】また、２）培養装置１の培養槽２として、内径２５０ｍｍ、高さ４８０ｍｍ、容量２０１の平底ジャーファーメンター（ＦＺ２０００：アルファーラバル製、スウェーデン）を用いた。平底ジャーファーメンター内の培地の温度制御は、ジャーファーメンター外側を覆っているジャケット内の管に通した水の温度を調節することにより行った。３）撹拌翼４ｂ…として、平板状に形成した、翼長Ａが８０ｍｍ、高さＢが１６０ｍｍの４枚翼を用い、回転軸４ａに対して、５° の傾斜角度をなして取り付け、５～１５r.p.m.の回転数で回転させた。４）通気チューブ３として、外径７ｍｍの多孔性フッ素樹脂チューブ（バイオット製、東京）５ｍを螺旋状に巻いて、平底ジャーファーメンター内に納めた。５）上記の平底ジャーファーメンターに、液面センサ（グラスライトセンサー：藤原製作所製、東京）、ｐＨ電極（４６５－５０－Ｓ７：インゴルト製、スイス）、およびＤＯ電極（φ２５ｍｍ－７０：インゴルト製、スイス）を取り付けた。

【００４９】さらに、６）細胞の培養は、培養装置１で本培養を行う前に、培養フラスコ、１１丸底フラスコ、および６１丸底フラスコを用いて前培養を行った。即ち、培養装置１で本培養を行う細胞は、培養フラスコ、

１１および６１丸底フラスコを用い、所定の細胞密度で接種した後、バッチ培養することにより増殖させた。以下、前培養に用いた上記３種類のフラスコについて説明する。

【００５０】培養フラスコ（図示せず）として、容量１５０ｍｌの静置式組織培養フラスコ（Ｔ－フラスコ：コーニング製、米国）を用いた。

【００５１】図３に示すように、１１丸底フラスコとして、内径１００ｍｍ、高さ２００ｍｍのガラス製スピナーフラスコ（柴田ハリオガラス製、東京）２２を用いた。このスピナーフラスコ２２における撹拌装置２４の撹拌翼２４ｂ…として、平板状に形成した翼長A_1が４０ｍｍ、高さB_1が２０ｍｍの２枚翼を用いた。通気方法は、フラスコ内のガスを置換する上面通気を行った。また、スピナーフラスコ２２に、図示しないｐＨ電極（４０５－ＤＰＡＳ－Ｋ８Ｓ／３２５：インゴルト製、スイス）を取り付けた。さらに、スピナーフラスコ２２内の培地の温度制御は、スピナーフラスコ２２外側を覆っている水槽（図示せず）の水温を調節することにより行った。

【００５２】図４に示すように、６１丸底フラスコとして、内径１９０ｍｍ、高さ２５０ｍｍのガラス製丸底ジャーファーメンター（柴田ハリオガラス製、東京）３２を用いた。この丸底ジャーファーメンター３２における撹拌装置３４の撹拌翼３４ｂ…として、平板状に形成した翼長A_2が４０ｍｍ、高さB_2が８０ｍｍの４枚翼を用い、回転軸３４ａに対して、５°の傾斜角度をなして取り付けた。チューブ通気を行う通気チューブ３３として、外径７ｍｍの多孔性フッ素樹脂チューブ（バイオット製、東京）３ｍを螺旋状に巻いて、丸底ジャーファーメンター３２内に納めた。また、上記の丸底ジャーファーメンター３２に、ｐＨ電極（４０５－ＤＰＡＳ－Ｋ８Ｓ／３２５：インゴルト製、スイス）３９ａ、およびＤＯ電極（φ１４ｍｍ：丸菱バイオエンジ製、東京）３９ｂを取り付けた。さらに、丸底ジャーファーメンター３２内の培地の温度制御は、ジャーファーメンター３２外側を覆っている水槽（図示せず）の水温を調節することにより行った。尚、撹拌装置３４の撹拌速度やｐＨ等の培養環境は、図示しない制御装置（ＭＣＴ－３Ｓ：丸菱バイオエンジ製、東京）を用いて制御した。

【００５３】そして、上記の１）～６）の条件下で、細胞を所定の細胞密度で平底ジャーファーメンター（培養槽２）内のＳ－ＲＤＦ－ＩＴＥＳ培地（培地１０）中に懸濁し、本培養を開始した。また、細胞の増殖に伴い、遠心分離機１１を用いた培地交換または連続潅流を行い培養を継続した。結果を実施例１～３として以下に示す。但し、本発明の培養方法は、実施例１～３に限定されるものではない。

【００５４】〔実施例１〕細胞として、マウスミエローマＰ３Ｘ６３Ａｇ８．６５３とヒトリンパ球との細胞融合により得られたヒトＩｇ（免疫グロブリン）Ｍ産生ヒト・マウスハイブリドーマ細胞株を用いた。

【００５５】先ず、細胞を、Ｔ－フラスコ内の培地７０ｍｌに、細胞密度１．０×１０^{5}個／ｍｌとなるように接種して懸濁し、二酸化炭素濃度５％の雰囲気下、３７℃で静置すると、培養開始後３日目に、細胞密度７．４×１０^{5}個／ｍｌに増殖した。また、培養終了時での抗体（目的物、ヒトＩｇＭ）濃度は２８ｍｇ／ｌであった。

【００５６】次に、Ｔ－フラスコ内で増殖した細胞を、スピナーフラスコ２２内の培地５００ｍｌに、細胞密度１．０×１０^{5}個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、３０ｒ.ｐ.ｍ.の回転数で撹拌すると、培養開始後３日目に、細胞密度６．６×１０^{5}個／ｍｌに増殖した。また、培養終了時での抗体濃度は２５ｍｇ／ｌであった。

【００５７】さらに、スピナーフラスコ２２内で増殖した細胞を、丸底ジャーファーメンター３２内の培地５１に、細胞密度１．０×１０^{5}個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１３ｒ.ｐ.ｍ.の回転数で撹拌すると、培養開始後３日目に、細胞密度７．９×１０^{5}個／ｍｌに増殖した。また、培養終了時での抗体濃度は３０ｍｇ／ｌであった。

【００５８】上記のようにして前培養した細胞を、平底ジャーファーメンター内の培地１５１に、細胞密度１．８×１０^{5}個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１０ｒ.ｐ.ｍ.の回転数で撹拌して本培養した。また、多孔性フッ素樹脂チューブ内の通気速度を２００～５００ｍｌ／ｍｉｎ．とし、本培養開始時はチューブ内に空気を通気して溶存酸素濃度を２～６ｐｐｍに保ち、細胞が増殖するに従い、空気中の酸素分圧を増加させて溶存酸素濃度を０．５～３ｐｐｍに制御した。すると、本培養開始後３日目に、細胞密度６．８×１０^{5}個／ｍｌに増殖した。この時点で４時間潅流を行い、培地を交換し、さらに、本培養開始後４日目以降は連続的に潅流を行った。尚、培地の交換率（潅流比）は、平底ジャーファーメンター内の培地液量（１５１）に対し、１～１．３回／日とした。また、本培養期間中、培地中のグルコース濃度を１ｇ／ｌ以上、乳酸濃度を２．７ｇ／ｌ以下にそれぞれ制御した。

【００５９】上記のようにして細胞を１５日間、本培養すると、表２に示すような細胞密度に増殖し、本培養開始後１３日目に、細胞密度７．７×１０^{6}個／ｍｌとなった。また、細胞が増殖するに伴い、表２に示すように抗体濃度も増加し、本培養開始後１２日目に、抗体濃度は１０５ｍｇ／ｌとなった。

【００６０】

【表２】

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培養日数(日)	細胞密度(個／ml)	抗体濃度(mg／l)
1	1．8×10^5	－
3	6．8×10^5	－
4	7．4×10^5	3
5	1．4×10^6	8
6	2．0×10^6	30
7	2．3×10^6	41
8	3．1×10^6	52
9	3．7×10^6	62
10	5．1×10^6	75
11	5．9×10^6	69
12	6．8×10^6	105
13	7．7×10^6	76
14	7．5×10^6	82
15	7．5×10^6	72

【００６１】以上の結果から、上記構成の培養装置１および培養方法は、細胞の増殖や目的物の生産性向上を促すことがわかる。即ち、上記構成の培養装置１および培養方法により、細胞に損傷等を与えずに増殖させることが可能であると共に、細胞を培養槽内で大量かつ高密度に培養して、目的物である抗体を安定して効率的に大量生産することが可能であることがわかる。

【００６２】〔実施例２〕細胞として、マウスミエローマＰ３Ｘ６３Ａｇ８．６５３とマウスリンパ球との細胞融合により得られたマウスＩｇＧ産生マウスハイブリドーマ細胞株を用いた。

【００６３】先ず、細胞を、Ｔ－フラスコ内の培地７０ｍｌに、細胞密度１．１×１０^5 個／ｍｌとなるように接種して懸濁し、二酸化炭素濃度５％の雰囲気下、３７℃で静置すると、培養開始後４日目に、細胞密度１．１５×１０^6 個／ｍｌに増殖した。細胞の倍加時間は２８．１時間であった。

【００６４】次に、Ｔ－フラスコ内で増殖した細胞を、スピナーフラスコ２２内の培地５００ｍｌに、細胞密度２．７×１０^5 個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、２０r.p.m.の回転数で撹拌すると、培養開始後３日目に、細胞密度８．２×１０^5 個／ｍｌに増殖した。細胞の倍加時間は４４．６時間であった。

【００６５】さらに、スピナーフラスコ２２内で増殖した細胞を、丸底ジャーファーメンター３２内の培地５１に、細胞密度３．１×１０^5 個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１２r.p.m.の回転数で撹拌すると、培養開始後２日目に、細胞密度１．０５×１０^6 個／ｍｌに増殖した。細胞の倍加時間は２７．０時間であった。

【００６６】上記のようにして前培養した細胞を、平底ジャーファーメンター内の培地１４．５１に、細胞密度２．９×１０^5 個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１０r.p.m.の回転数で撹拌して本培養した。また、多孔性フッ素樹脂チューブ内の通気速度を２００～５００ｍｌ／ｍｉｎ．とし、本培養開始時はチューブ内に空気を通気して溶存酸素濃度を２～６ｐｐｍに保ち、細胞が増殖するに従い、空気中の酸素分圧を増加させて溶存酸素濃度を０．５～３ｐｐｍに制御した。すると、本培養開始後４日目に、細胞密度１．０８×１０^6 個／ｍｌに増殖した。この時点で３時間灌流を行い、培地を交換した。また、本培養開始後５日目に、細胞密度１．３３×１０^6 個／ｍｌに増殖した。この時点で３時間灌流を行い、培地を交換し、さらに、本培養開始後６日目以降は連続的に灌流を行った。尚、培地の交換率（灌流比）は、平底ジャーファーメンター内の培地液量（１４．５１）に対し、１．５～２．０回／日とした。また、本培養期間中、培地中のグルコース濃度を１．５ｇ／ｌ以上、乳酸濃度を２．３ｇ／ｌ以下にそれぞれ制御した。

【００６７】上記のようにして細胞を１８日間、本培養すると、表３に示すような細胞密度に増殖し、本培養開始後１８日目に、細胞密度３．４×１０^6 個／ｍｌとなった。

【００６８】

【表３】

培養日数(日)	細胞密度(個／ml)
1	2．9×10^5
4	1．1×10^6
5	1．3×10^6
6	1．5×10^6
7	1．5×10^6
8	1．8×10^6
9	1．7×10^6
10	1．8×10^6
11	1．7×10^6
12	1．8×10^6
13	2．0×10^6
14	2．2×10^6
15	2．3×10^6
16	2．7×10^6
17	2．7×10^6
18	3．4×10^6

【００６９】以上の結果から、上記構成の培養装置１および培養方法は、細胞の増殖を促すことがわかる。即ち、上記構成の培養装置１および培養方法により、細胞に損傷等を与えずに増殖させることが可能であると共に、細胞を培養槽内で大量かつ高密度に培養することが可能であることがわかる。

【００７０】〔実施例３〕細胞として、ナマルバ細胞を宿主とするヒトＩｇＭ産生組み換え細胞を用いた。

【００７１】先ず、細胞を、Ｔ－フラスコ内の培地７０ｍｌに、細胞密度２．０×１０^5 個／ｍｌとなるように接種して懸濁し、二酸化炭素濃度５％の雰囲気下、３７

℃で静置すると、培養開始後４日目に、細胞密度９．４×１０⁵ 個／ｍｌに増殖した。細胞の倍加時間は４３．７時間であった。また、培養終了時での抗体（目的物、ヒトＩｇＭ）濃度は５．８ｍｇ／ｌであった。

【００７２】次に、Ｔ－フラスコ内で増殖した細胞を、スピナーフラスコ２２内の培地５００ｍｌに、細胞密度２．１×１０⁵ 個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１５ｒ.ｐ.ｍ.の回転数で撹拌すると、培養開始後４日目に、細胞密度１．２７×１０⁶ 個／ｍｌに増殖した。細胞の倍加時間は３３．８時間であった。また、培養終了時での抗体濃度は３ｍｇ／ｌであった。

【００７３】さらに、スピナーフラスコ２２内で増殖した細胞を、丸底ジャーファーメンター３２内の培地５１に、細胞密度２．６×１０⁵ 個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１５ｒ.ｐ.ｍ.の回転数で撹拌すると、培養開始後５日目に、細胞密度７．９×１０⁵ 個／ｍｌに増殖した。細胞の倍加時間は７１．６時間であった。また、培養終了時での抗体濃度は３．９ｍｇ／ｌであった。

【００７４】上記のようにして前培養した細胞を、平底ジャーファーメンター内の培地１５．８１に、細胞密度２．３×１０⁵ 個／ｍｌとなるように接種して懸濁し、３７℃、ｐＨ６．８～７．１、１０ｒ.ｐ.ｍ.の回転数で撹拌して本培養した。また、多孔性フッ素樹脂チューブ内の通気速度を２００～５００ｍｌ／ｍｉｎ．とし、本培養開始時はチューブ内に空気を通気して溶存酸素濃度を２～６ｐｐｍに保ち、細胞が増殖するに従い、空気中の酸素分圧を増加させて溶存酸素濃度を０．５～３ｐｐｍに制御した。すると、本培養開始後３日目に、細胞密度９．２×１０⁵個／ｍｌに増殖した。この時点で３時間潅流を行い、培地を交換した。本培養開始後５日目に、細胞密度１．７×１０⁶ 個／ｍｌに増殖した。この時点で３時間潅流を行い、培地を交換した。本培養開始後６日目に、細胞密度２．７×１０⁶個／ｍｌに増殖した。この時点で３時間潅流を行い、培地を交換した。本培養開始後７日目に、細胞密度４．１×１０⁶ 個／ｍｌに増殖した。この時点で３時間潅流を行い、培地を交換し、さらに、本培養開始後８日目以降は連続的に潅流を行った。尚、培地の交換率（潅流比）は、平底ジャーファーメンター内の培地液量（１５．８ｌ）に対し、１．５～５回／日とした。また、本培養期間中、培地中のグルコース濃度を１．５ｇ／ｌ以上、乳酸濃度を２．５ｇ／ｌ以下にそれぞれ制御した。

【００７５】上記のようにして細胞を２１日間、本培養すると、表４に示すような細胞密度に増殖し、本培養開始後２０日目に、細胞密度１．８×１０⁷ 個／ｍｌとなった。また、細胞が増殖するに伴い、表４に示すように抗体濃度も増加し、本培養開始後１１日目以降の抗体濃度は、１０～１９ｍｇ／ｌと安定した。

【００７６】

【表４】

培養日数（日）	細胞密度（個／ｍｌ）	抗体濃度（ｍｇ／ｌ）
1	2．3×10⁵	－
3	9．2×10⁵	4
4	9．6×10⁵	－
5	1．7×10⁶	13
6	2．7×10⁶	－
7	4．1×10⁶	15
8	4．7×10⁶	－
9	7．0×10⁶	－
10	8．4×10⁶	－
11	9．6×10⁶	14
12	1．2×10⁷	15
13	1．4×10⁷	17
14	1．5×10⁷	19
15	1．6×10⁷	12
16	1．6×10⁷	12
17	1．6×10⁷	15
18	1．7×10⁷	10
19	1．7×10⁷	17
20	1．8×10⁷	11
21	1．8×10⁷	14

【００７７】以上の結果から、上記構成の培養装置１および培養方法は、細胞の増殖や目的物の生産性向上を促すことがわかる。即ち、上記構成の培養装置１および培養方法により、細胞に損傷等を与えずに増殖させることが可能であると共に、細胞を培養槽内で大量かつ高密度に培養して、目的物である抗体を安定して効率的に大量生産することが可能であることがわかる。

【００７８】尚、上記の実施例１～３は、本発明の培養方法および培養装置を用いた細胞培養の一例を示すものであり、培養槽２等の寸法、前培養の方法および装置、培地１０の成分組成、細胞の種類、培地の温度やｐＨ等を始めとする培養環境等は、勿論、上記の実施例１～３に示した数値や種類等に限定されるものではなく、必要に応じて適宜変更が可能である。また、細胞の培養日数（期間）は、勿論、上記の実施例１～３に示した日数で終了もしくは打ち切られるものではなく、本発明の培養方法および培養装置を用いて、細胞を数ヵ月あるいは数年に渡って連続培養することが可能である。

【００７９】

【発明の効果】請求項１記載の発明の動物細胞の培養方法は、以上のように、回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段で上記の細胞培養液を撹拌する方法である。

【００８０】これにより、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが

可能となるという効果を奏する。

【００８１】請求項２記載の発明の動物細胞の培養方法は、以上のように、請求項１記載の動物細胞の培養方法において、回転速度を撹拌翼の外周部分で８００ｃｍ／分以下とする方法である。

【００８２】これにより、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内でより大量かつ高密度に培養することが可能となるという効果を奏する。

【００８３】請求項３記載の発明の動物細胞の培養方法は、以上のように、請求項１または請求項２記載の動物細胞の培養方法において、細胞培養液中に少なくとも多孔性チューブを有する酸素供給手段でチューブ通気を行って動物細胞に酸素を供給すると共に、分離手段で細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還し、かつ、培養液供給手段で培養槽に新しい細胞培養液を供給する方法である。

【００８４】これにより、培養槽内を動物細胞にとって最適の条件に保つことができると共に、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが可能となるという効果を奏する。

【００８５】請求項４記載の発明の動物細胞の培養装置は、以上のように、回転半径が上記の培養槽内径の１／４以上、３／８以下の、撹拌翼１枚の面積が上記の培養槽の細胞培養液部の縦方向断面積の１／１５以上となるような大きさに形成された撹拌翼を有する撹拌手段を備え、この撹拌手段により上記の細胞培養液を撹拌する構成である。

【００８６】これにより、動物細胞に、撹拌による剪断力等の物理的刺激による損傷や破壊等の悪影響を与えずに、培養槽内で大量かつ高密度に培養することが可能となるという効果を奏する。

【００８７】請求項５記載の発明の動物細胞の培養装置は、以上のように、請求項４記載の動物細胞の培養装置において、回転速度が撹拌翼の外周部分で８００ｃｍ／分以下である構成である。

【００８８】これにより、動物細胞に、撹拌による剪断力等の物理的刺激による損傷や破壊等の悪影響を与えずに、培養槽内でより大量かつ高密度に培養することが可能となるという効果を奏する。

【００８９】請求項６記載の発明の動物細胞の培養装置は、以上のように、請求項４または請求項５記載の動物細胞の培養装置において、少なくとも多孔性チューブを有し、細胞培養液中にチューブ通気を行って動物細胞に酸素を供給する酸素供給手段と、細胞培養液と動物細胞とを遠心分離し、細胞培養液を培養槽外に抜き取る一方、動物細胞を培養槽に返還する分離手段と、培養槽に新しい細胞培養液を供給する培養液供給手段とを備えている構成である。

【００９０】これにより、培養槽内を動物細胞にとって最適の条件に保つことができると共に、動物細胞が撹拌による剪断力等の物理的刺激により損傷や破壊等の悪影響を受けることはなく、培養槽内で大量かつ高密度に培養することが可能となるという効果を奏する。

【図面の簡単な説明】

【図１】本発明の一実施例における培養装置の構成を示すブロック図である。

【図２】上記培養装置における撹拌翼の大きさを示す説明図である。

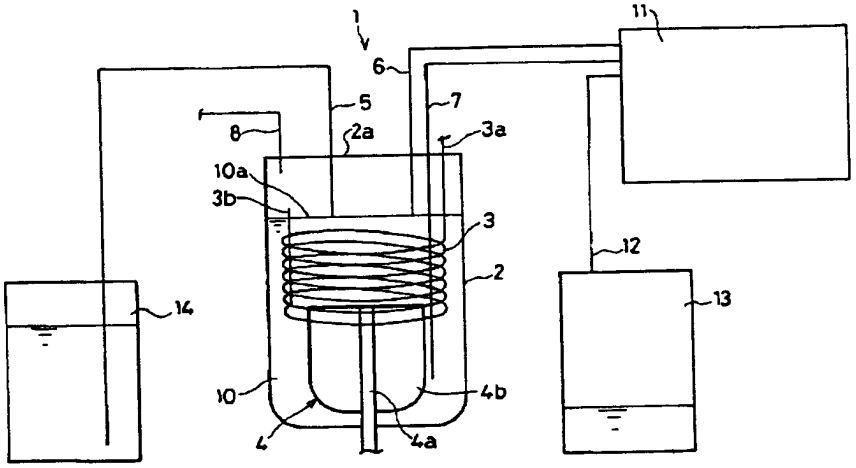
【図３】前培養に用いるスピナーフラスコの説明図である。

【図４】前培養に用いる丸底ジャーファーメンターの説明図である。

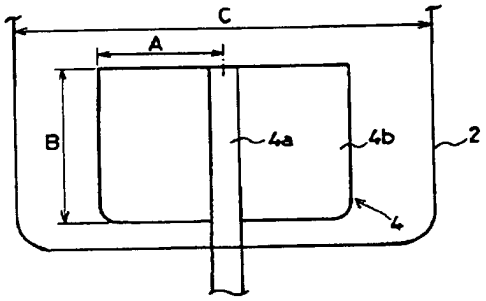
【符号の説明】

１　培養装置
２　培養槽
３　通気チューブ（酸素供給手段）
４　撹拌装置（撹拌手段）
４ｂ　撹拌翼
５　培地注入管（培養液供給手段）
６　培地抜き取り管（分離手段）
７　細胞返却管（分離手段）
１０　培地（細胞培養液）
１１　遠心分離機（分離手段）
１３　目的物貯液槽（分離手段）
１４　培地貯液槽（培養液供給手段）
Ａ　翼長（即ち、回転半径）
Ｂ　高さ
Ｃ　内径

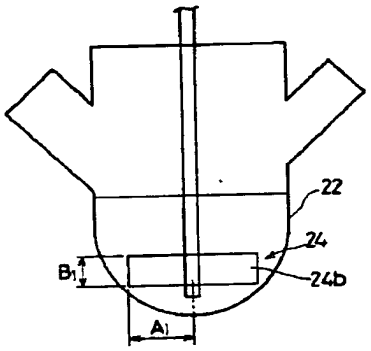
【図１】


1
11
6
5
7
8
2a
3a
10a
3b
3
12
2
14
13
10
4b
4
4a

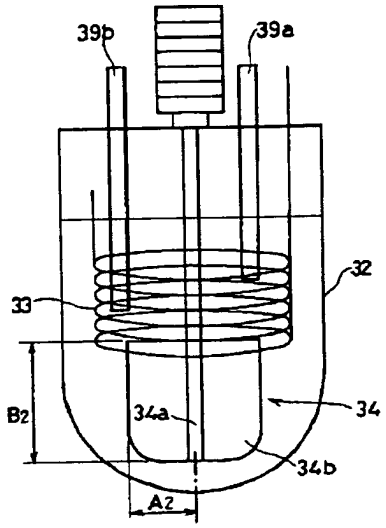
【図２】


C
A
B
4a
4b
2
4

【図３】


22
24
B1
24b
A1

【図４】


39b
39a
32
33
B2
34a
34
34b
A2

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